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(54) Title: DEVICE FOR IMPLEMENTING AN ANALYSIS PACK, ANALYSIS PACK AND METHOD USING SAME

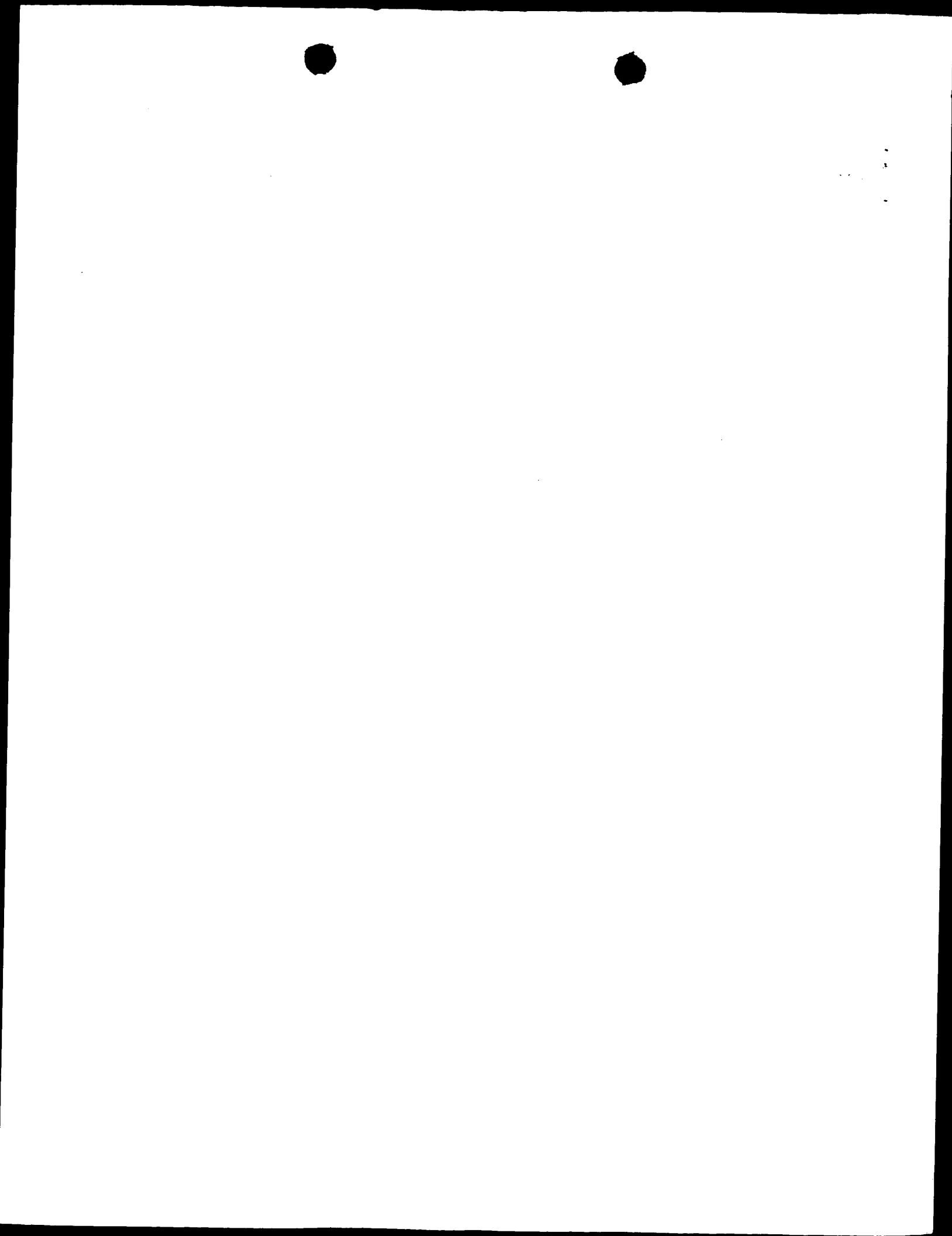
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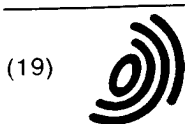
(57) Abstract: The invention concerns a device for implementing an analysis pack wherein reaction chains and fluid transfers are performed under the effect of control means internal to said pack, said pack comprising at least two parallel reaction chains, each chain consisting of at least two fluid transfer in series. The invention also concerns an analysis pack and the method using the device and the pack. The device is characterised in that it comprises at least one actuator per reaction chain, and all the actuators are at a constant distance from one another and preferably the actuators are equidistant. The invention is particularly applicable in the field of microfluidics used in biology.

(57) Abrégé: La présente invention concerne un dispositif de mise en oeuvre d'une carte d'analyse au sein de laquelle des chaînes réactionnelles et des transferts de fluides sont réalisés sous l'effet de moyens de contrôle internes à la carte; ladite carte comporte au moins deux chaînes réactionnelles en parallèle, chaque chaîne étant constituée d'au moins deux transferts de fluides en série. L'invention concerne également une carte d'analyse et le procédé mettant en oeuvre le dispositif et la carte. Le dispositif se caractérise par le fait qu'il comporte au moins un actionneur par chaîne réactionnelle, et que tous les actionneurs sont à une distance constante les uns des autres et préférentiellement les actionneurs sont équidistants. L'invention trouve une application préférentielle dans le domaine de la micro-fluidique appliquée à la biologie.



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(54) Dual chamber disposable reaction vessel for amplification reactions, reaction processing station therefor, and methods of use

(57) A reaction vessel for a nucleic acid amplification reaction has a first chamber containing an amplification reagent mix, a second chamber containing an amplification enzyme, and a fluid channel or chamber connecting the first and second chambers together. A fluid sample is introduced into the first chamber. After a denaturation and primer annealing process has occurred in the first chamber, the fluid chamber is opened to allow the solution of the reagent and fluid sample to flow into the second chamber. The second chamber is maintained at an optimal temperature for the amplification reaction.

A station is described for processing test strips incorporating the reaction vessels. The station includes temperature and vacuum control subsystems to maintain proper temperatures in the reaction vessel and effectuate the transfer of the fluid from one chamber to the other in an automated fashion without human intervention.

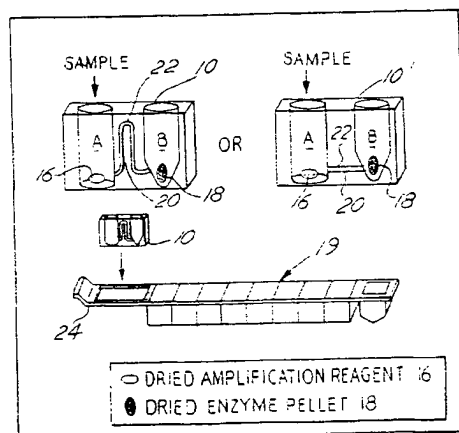


FIG.3

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## Description

This invention relates to the field of the equipment and methods used for performing nucleic acid amplification reactions. More specifically, the invention relates to a novel disposable dual chamber reaction vessel for a nucleic acid amplification reaction and a station for conducting the reaction in the reaction vessel.

Nucleic acid based amplification reactions are now widely used in research and clinical laboratories for the detection of genetic and infectious diseases. The currently known amplification schemes can be broadly grouped into two classes, based on whether, after an initial denaturing step (typically performed at a temperature of  $\geq 65$  degrees C) for DNA amplifications or for RNA amplifications involving a high amount of initial secondary structure, the reactions are driven via a continuous cycling of the temperature between the denaturation temperature and a primer annealing and amplification synthesis (or polymerase activity) temperature, or whether the temperature is kept constant throughout the enzymatic amplification process. Typical cycling reactions are the Polymerase and Ligase Chain Reaction (PCR and LCR, respectively). Representative isothermal reaction schemes are NASBA (Nucleic Acid Sequence Based Amplification), Transcription Mediated Amplification (TMA), and Strand Displacement Amplification (SDA). In the isothermal reactions, after the initial denaturation step (if required), the reaction occurs at a constant temperature, typically a lower temperature at which the enzymatic amplification reaction is optimized.

Prior to the discovery of thermostable enzymes, methodologies that used temperature cycling were seriously hampered by the need for dispensing fresh polymerase after each denaturation cycle, since the elevated temperature required for denaturation inactivated the polymerase during each cycle. A considerable simplification of the PCR assay procedure was achieved with the discovery of the thermostable Taq polymerase (from *Thermophilus aquaticus*). This improvement eliminated the need to open amplification tubes after each amplification cycle to add fresh enzyme. This led to the reduction of both the contamination risk and the enzyme-related costs. The introduction of thermostable enzymes has also allowed the relatively simple automation of the PCR technique. Furthermore, this new enzyme allowed for the implementation of simple disposable devices (such as a single tube) for use with temperature cycling equipment.

TMA requires the combined activities of at least two (2) enzymes for which no optimal thermostable variants have been described. For optimal primer annealing in the TMA reaction, an initial denaturation step (at a temperature of  $\geq 65$  degrees C) is performed to remove secondary structure of the target. The reaction mix is then cooled down to a temperature of 42 degrees C to allow primer annealing. This temperature is also the optimal reaction temperature for the combined activities of T7

RNA polymerase and Reverse Transcriptase (RT), which includes an endogenous RNase H activity or is alternatively provided by another reagent. The temperature is kept at 42 degrees C throughout the following isothermal amplification reaction. The denaturation step, which precedes the amplification cycle, however forces the user to add the enzyme after the cool down period in order to avoid inactivation of the enzymes. Therefore, the denaturation step needs to be performed separately from the amplification step.

In accordance with present practice, after adding the test or control sample or both to the amplification reagent mix (typically containing the nucleotides and the prime), the tube is subject to temperatures  $\geq 65$  degrees C and then cooled down to the amplification temperature of 42 degrees C. The enzyme is then added manually to start the amplification reaction. This step typically requires the opening of the amplification tube. The opening of the amplification tube to add the enzyme or the subsequent addition of an enzyme to an open tube is not only inconvenient, it also increases the contamination risk.

The present invention avoids the inconvenience and contamination risk described above by providing a novel dual chamber or "binary" reaction vessel, a reaction processing station therefor, and methods of use that achieve the integration of the denaturation step with the amplification step without the need for a manual enzyme transfer and without exposing the amplification chamber to the environment. The contamination risks from sample to sample contamination within the processing station are avoided since the amplification reaction chamber is sealed and not opened to introduce the patient sample to the enzyme. Contamination from environmental sources is avoided since the amplification reaction chamber remains sealed. The risk of contamination in nucleic acid amplification reactions is especially critical since large amounts of the amplification product are produced. The present invention provides a reaction chamber design that substantially eliminates these risks.

In a preferred form of the invention, a dual chamber reaction vessel is provided which comprises a single or unit dose of reagents for a reaction requiring differential heat and containment features, such as a nucleic acid amplification reaction (for example, TMA reaction) packaged ready for use. The dual chamber reaction vessel is designed as a single use disposable unit. The reaction vessel is preferably integrally molded into a test strip having a set of wash and reagent wells for use in an amplification product detection station. Alternatively, the reaction vessel can be made as a stand alone unit with flange or other suitable structures for being able to be installed in a designated space provided in such a test strip.

In the dual chamber reaction vessel, two separate reaction chambers are provided in a preferred form of the invention. The two main reagents for the reaction

are stored in a spatially separated fashion. One chamber has the heat stable sample/amplification reagent (containing primers, nucleotides, and other necessary salts and buffer components), and the other chamber contains the heat labile enzymatic reagents, e.g., T7 and RT.

The two chambers are linked to each other by a fluid channel extending from the first chamber to the second chamber. A means is provided for controlling or allowing the flow of fluid through the fluid channel from the first chamber to the second chamber. In one embodiment, a membrane is molded into the reaction vessel that seals off the fluid channel. A reciprocable plunger or other suitable structure is provided in the reaction vessel (or in the processing station) in registry with the membrane. Actuation of the plunger causes a breaking of the membrane seal, allowing fluid to flow through the fluid channel. Differential pressure between the two chambers assists in transferring the patient or clinical or control sample through the fluid channel from the first chamber to the second chamber. This can be accomplished by applying pressure to the first chamber or applying vacuum to the second chamber.

Other types of fluid flow control means are contemplated, such as providing a valve in the fluid channel. Several different valve embodiments are described.

In use, the fluid sample is introduced into the first chamber and the first chamber is heated to a denaturation temperature (e.g., 95 degrees C). After the amplification reagents in the first chamber have reacted with the fluid sample and the denaturation process has been completed, the first chamber is quickly cooled to 42 degrees C for primer annealing. The two chambers of the reaction vessel are not in fluid communication with each other prior to completion of the denaturation and cooling step. After these steps are complete, the means for controlling the flow of fluid is operated to allow the reaction solution to pass through the fluid channel from the first chamber to the second chamber. For example, the valve in the fluid channel is opened and the fluid sample is directed into the second chamber either by pressure or vacuum techniques. The reaction solution is then brought into contact with the amplification enzyme(s) (e.g., T7 and/or RT) and the enzymatic amplification process proceeds in the second chamber at 42 degrees C.

In a preferred embodiment, after completion of the reaction, a SPR® (solid phase receptacle) pipette-like device is introduced into the second chamber. Hybridization, washing and optical analysis then proceeds in accordance with well known techniques in order to detect the amplification products.

An integrated stand-alone processing station for processing a reaction in the dual chamber reaction vessel in accordance with presently preferred embodiments of the invention is described. The processing station includes a tray for carrying in proper alignment a plurality of test strips, a temperature control subassembly for maintaining the two chambers of the reaction vessel at

the proper temperatures, a mechanism to open the fluid channel connecting the two chambers together, and a vacuum subassembly for providing vacuum to the second chamber to draw the fluid sample from the first chamber into the second chamber.

Presently preferred embodiments of the invention will be described in conjunction with the appended drawings, wherein like reference numerals refer to like elements in the various views, and in which:

Figure 1 is a schematic representation of a disposable dual chamber reaction vessel and the heating steps associated therewith to perform an isothermal amplification reaction, i.e., a TMA reaction, in accordance with one possible embodiment of the invention;

Figure 2 is a schematic representation of alternative form of the invention in which two separate reaction chambers are combined to form a dual chamber reaction vessel;

Figure 3 is a schematic representation of two alternative embodiments of a dual chamber reaction vessel that are snapped into place in a test strip for processing with a solid phase receptacle and optical equipment in accordance with a preferred embodiment of the invention;

Figure 4 is a schematic representation of an alternative embodiment of a dual chamber reaction vessel formed from two separate chambers that are combined in a manner to permit a fluid sample in one chamber to be transferred to the other chamber, with the combined dual chamber vessel placed into a test strip such as illustrated in Figure 3;

Figure 5 is a detailed perspective view of a disposable test strip in which one embodiment of the dual chamber reaction vessel is integrally molded into the test strip at the left-hand end of the test strip;

Figure 6 is detailed perspective view of the disposable test strip of Figure 5 as seen from below;

Figure 7 is a cross section of the disposable test strip of Figures 5 and 6, showing a plunger having a chisel-like tip that is used to pierce a membrane in a fluid channel connecting the two chambers together to thereby allow the fluid to pass from the first chamber into the second chamber;

Figure 8 is a perspective view of the left hand end of the test strip of Figures 5-7 shown enlarged in order to better illustrate the dual chamber reaction vessel;

Figure 9 is a detailed perspective view of a disposable test strip of Figure 5 as seen from below shown greatly enlarged, and with the cap covering the base of the first chamber and intermediate chamber removed;

Figure 10 is a top plan view of the dual chamber reaction vessel of Figures 5-9 shown enlarged;

Figure 11 is a detailed cross section of the dual chamber reaction vessel with the lower cap re-

moved as in Figure 9, and with the plunger removed:

Figure 12 is a detailed cross section of the dual chamber reaction vessel with the lower cap and plunger installed as they would be in use:

Figure 13 is a perspective view of the plunger of Figure 12:

Figure 14 is another perspective view of the plunger:

Figure 15 is an elevational view of the plunger:

Figure 16 is a perspective view of the cap that covers the base of the first chamber and the intermediate chamber of the reaction vessel of Figures 8 and 9:

Figure 17 is a cross-section of the cap of Figure 16:

Figure 18 is a perspective view of the base of cap of Figure 16:

Figure 19 is a perspective view of a stand-alone disposable dual chamber reaction vessel that is designed to snap into the test strip of the type shown in Figure 5 in the manner suggested in Figure 4:

Figure 20 is a perspective view of the stand-alone disposable dual chamber reaction vessel of Figure 19, with a lower cap as shown in Figures 16-18 removed:

Figure 21 is perspective view of an alternative construction of the stand-alone disposable dual chamber reaction vessel of Figure 19:

Figure 22 is a cross-sectional view of the embodiment of Figure 21:

Figure 23 is a cross-sectional view of the embodiment of Figure 21 showing the action of the helical thimble valve being deformed by a vacuum plunger and the flow of fluid sample from the first chamber into the second chamber:

Figure 24 is a perspective view of the helical thimble valve of Figures 22 and 23:

Figure 25 is a sectional view of the embodiment of Figure 21 showing the flow of fluid through the device from the first chamber into the second chamber:

Figure 26 is a perspective view of another embodiment of the disposable reaction chamber in accordance with the invention designed to snap into the test strip in the manner suggested in Figure 4:

Figure 27 is a cross-section of the embodiment of Figure 26, showing an enzyme plunger carrying an enzyme pellet for introduction into the amplification well:

Figure 28 is a cross-section of a test strip incorporating the embodiment of Figure 26:

Figures 29A-29C show the use of the test strip of Figure 28:

Figure 30 is a schematic representation of an embodiment of a dual chamber disposable reaction vessel in which a plunger is activated to increase the fluid pressure in the first reaction chamber to break a seal in a fluid channel connecting the first

chamber to the second chamber and force a reaction solution in the first chamber into the second chamber for the amplification reaction to take place:

Figure 31 is a perspective view of a stand-alone amplification processing station for the test strips having the dual chamber reaction vessels in accordance with a presently preferred form of the invention:

Figure 32 is a perspective view of one of the amplification modules of Figure 31, as seen from the rear of the module:

Figure 33 is a perspective view of the front of the module of Figure 32:

Figure 34 is another perspective view of the module of Figure 33:

Figure 35 is a detailed perspective view of a portion of the test strip holder and 95 degree C Peltier heating subsystems of the module of Figures 32-34:

Figure 36 is an isolated perspective view of the test strip holder of Figure 35, showing two test strips in accordance with Figure 5 installed in the test strip holder:

Figure 37 is a detailed perspective view of the test strip holder or tray of Figure 33:

Figure 38 is a block diagram of the electronics of the amplification processing station of Figure 33:

Figure 39 is a diagram of the vacuum subsystem for the amplification processing station of Figure 31:

Figure 40 is a graph of the thermal cycle of the station of Figure 31:

Figure 41 is a perspective view of another embodiment of a dual chamber reaction vessel that is suited for use with the test strip of Figure 3 and the reaction processing station of Figures 30-39:

Figure 42 is a vertical sectional view of the vessel of Figure 41 along the line 42-42 of Figure 41:

Figure 43 is a top view of the vessel of Figure 42:

Figure 44 is a detailed illustration of how the conduit and external constriction device work together in a first possible embodiment of the vessel of Figure 41:

Figure 45 is a detailed illustration of how the conduit and external constriction device work together in a second possible embodiment of the vessel of Figure 41:

Figures 46 is a schematic representation of a dual chamber reaction vessel in accordance with one possible embodiment of the invention, with the schematic representation corresponding, for example, to the embodiment of FIG. 41; and

Figure 47A-47F are schematic drawings showing the different stages of a process for transferring reagent solutions into the vessel and from the first chamber to the second chamber

A preferred form of the invention provides for a dual chamber or "binary" reaction vessel. The term "binary" refers to the characteristic of the vessel of storing in a spatially separated fashion at least two different rea-

gents, for example a heat stable sample/amplification reagent(s) containing, for example, primers and nucleotides in one chamber and heat labile enzyme(s) such as T7 and RT in the second chamber. The reagents within the two chambers are not in contact prior to completion of the denaturation and cooling steps. The first chamber is accessible via a pierceable membrane or other means so as to permit a patient or clinical or control sample(s) in liquid form to be added into the first chamber. The second chamber is sealed and contains the enzymatic components of the amplification reaction. The enzymatic components may be in several physical forms, such as liquid, pelletized, lyophilized, etc. After the contents of the first chamber is brought into contact with the second chamber, the reaction can then take place, such as in the second chamber.

In one possible form of the invention, the two chambers may be part of an integrated disposable unit. In another possible embodiment, the two chambers may be two distinct units which have complementary engaging surfaces or features that allow the two units to be combined into a single unit. In the first embodiment, where the two chambers are part of a unitary article, the unit must be made to prohibit the exchange of materials between the two chambers during shipping and prior to the denaturation (heating) step. In both embodiments, a mechanism is required by which the contents of the first chamber (the patient or test sample and amplification reagent(s) mix after denaturation and primer annealing) is brought into contact with the enzyme(s) in the second chamber. The mechanism operates to introduce the contents of the first chamber into the second chamber following the completion of the denaturation step and the cooling of the patient sample/amplification mix to the appropriate temperature for the enzymatic amplification reaction, e.g., 42 degrees C. Several different mechanisms are described in detail herein.

Figure 1 is a schematic representation of a disposable dual chamber reaction vessel 10 and the heating steps associated therewith to perform an isothermal reaction, i.e., a TMA reaction, in accordance with one possible embodiment of the invention. Chamber A contains the amplification reagents or mix, namely deoxynucleotides, primers,  $MgCl_2$  and other salts and buffer components. Chamber B contains the amplification enzyme(s) that catalyzes the amplification reaction, e.g., T7 and/or RT. After addition of the targets (or patient sample) into chamber A, heat is applied to chamber A to denature the DNA nucleic acid targets and/or remove RNA secondary structure. The temperature of chamber A is then quickly cooled down to allow primer annealing. Subsequently, the solution of chamber A is brought into contact with chamber B. Chambers A and B, now in fluid communication with each other, are then maintained at the optimum temperature for the amplification reaction, e.g., 42 degrees C. By spatially separating chamber A from chamber B, and applying the heat for denaturation to chamber A only, the thermolabile enzymes in cham-

ber B are protected from inactivation during the denaturation step.

Figure 2 is a schematic representation of an alternative form of the invention in which two separate reaction chambers 12 and 14 are combined to form a dual chamber reaction vessel 10. Like the embodiment of Figure 1, Chamber A is pre-loaded during a manufacturing step with an amplification reagent(s) or mix, namely nucleotides, primers,  $MgCl_2$  and other salts and buffer components. Chamber B is pre-loaded during manufacturing with the amplification enzyme(s) that catalyzes the amplification reaction, e.g., T7 and/or RT. Fluid sample is then introduced into chamber A. The sample is heated for denaturation of nucleic acids to 95 degrees C in chamber A. After cooling chamber A to 42 degrees C, the solution in chamber A is brought into contact with the enzymes in chamber B to trigger the isothermal amplification reaction.

If the reaction vessel is designed such that, after having brought the contents of chambers A and B into contact, the amplification chamber does not allow any exchange of materials with the environment, a closed system amplification is realized which minimizes the risk of contaminating the amplification reaction with heterologous targets or amplification products from previous reactions or the environment.

Figure 3 is a schematic representation of two alternative dual chamber reaction vessels 10 and 10' that are snapped into place in a test strip 19 for processing with a solid phase receptacle and optical equipment in accordance with a preferred embodiment of the invention. In the embodiments of Figure 3, a unidirectional flow system is provided. The sample is first introduced into chamber A for heating to the denaturation temperature. Chamber A contains the dried amplification reagent mix 16. After cooling, the fluid is transferred to chamber B containing the dried enzyme(s) 18 in the form of a pellet. Chamber B is maintained at 42 degrees C after the fluid sample is introduced into Chamber B. The amplification reaction takes place in Chamber B at the optimum reaction temperature (e.g., 42 degrees C). After the reaction is completed, the test strip 19 is then processed in a machine such as the VIDAS instrument commercially available from biomérieux Vitek, Inc., Hazelwood, Missouri, the assignee of the present invention. Persons of skill in the art are familiar with the VIDAS instrument.

The unidirectional flow features could be provided by a suitable one-way valve such as check valve 20 in the fluid conduit 22 connecting chambers A and B. The action of transferring the fluid from chamber A to chamber B could be by any of several possible methods, such as by introduction of fluid pressure in the solution in chamber A (such as by a piston), or applying a vacuum to chamber B to draw the solution through the fluid channel 22. Examples of these methods are described in detail below.

The steps of heating and cooling of chamber A

could be performed prior to the insertion of the dual chamber disposable reaction vessel 10 or 10' into the test strip 16. or, alternatively, suitable heating elements could be placed adjacent to the left hand end 24 of the test strip 19 in order to provide the proper temperature control of the reaction chamber A. The stand alone amplification processing station of Figures 31-40, described below, incorporates suitable heating elements and control systems to provide the proper temperature control for the reaction vessel 10.

Figure 4 is a schematic representation of an alternative embodiment of a dual chamber reaction vessel 10" formed from two separate interlocking vessels 10A and 10B that are combined in a manner to permit a fluid sample in one chamber to flow to the other, with the combined dual chamber vessel 10" placed into a test strip 19 such as described above in Figure 3. The fluid sample is introduced into chamber A, which contains the dried amplification reagent mix 16. Vessel A is then heated off-line to 95 degrees C, then cooled to 42 degrees C. The two vessels A and B are brought together by means of a conventional snap fit between complementary locking surfaces on the tube projection 26 on chamber B and the recessed conduit 28 on chamber A. The mixing of the sample solution from chamber A with the enzyme(s) from chamber B occurs since the two chambers are in fluid communication with each other, as indicated by the arrow 30. The sample can then be amplified in the combined dual chamber disposable reaction vessel 10" off-line, or on-line by snapping the combined disposable vessel 10" into a modified VIDAS strip. The VIDAS instrument could perform the detection of the amplification reaction products in known fashion.

Figure 5 is a detailed perspective view of a modified disposable test strip 19 similar to that used in the VIDAS instrument in which a dual chamber reaction vessel 10 comprising a first chamber 32 and a second chamber 34 is integrally molded into the test strip 19 at the left-hand end 24 of the test strip. The test strip 19 includes a plurality of wells to the right of the dual chamber reaction vessel 10. These wells include a probe well 36, a hybridization well 38, an empty well 40, four wash buffer wells 42, 44, 46 and 48, and a well 50 for containing a bleach solution. A substrate cuvette 52 is inserted into the opening 52 at the right hand end 54 of the strip for performance of optical analysis. The test strip 19 is used in conjunction with a SPR®, not shown in the drawings, which is used to draw a fluid sample out of the amplification well 34. The SPR is then dipped into the other wells 36 - 50 during the test procedure in known fashion to perform the analysis, for example as performed in the commercially available VIDAS instrument.

Figure 6 is a detailed perspective view of a disposable test strip of Figure 5 as seen from below. Figure 7 is a cross section of the disposable test strip of Figures 5 and 6, showing a plunger 56 having a chisel-like tip at the lower end thereof that is used to pierce a membrane in a fluid channel connecting the two chambers 32 and

34 together to thereby allow the fluid to pass from the first chamber 32 into the second or amplification chamber 34.

Figure 8 is a perspective view of the left hand end of the test strip of Figures 5-7 shown enlarged in order to better illustrate the dual chamber reaction vessel 10. Figure 9 is a detailed perspective view of a disposable test strip of Figure 5 as seen from below shown greatly enlarged, and with a cap 60 (Figure 12) covering the base of the first chamber and the intermediate chamber or fluid channel removed to better illustrate the structure of the device.

Figure 10 is a top plan view of the dual chamber reaction vessel of Figures 5-9 shown enlarged. Figure 11 is a detailed cross-section of the dual chamber reaction vessel with the lower cap removed as in Figure 9, and with the plunger removed. Figure 12 is a detailed cross section of the dual chamber reaction vessel with the lower cap 60 and plunger 56 installed as they would be in use.

Referring to Figures 5-12, the test strip 19 includes a molded body 62 that defines the walls of a reaction vessel 10. The vessel 10 includes a first chamber 32 in which a dried amplification reagent mix is placed at the bottom of the chamber 32 during manufacturing of the test strip 19. Polypropylene is a suitable material for use in molding the device 10 and test strip 19, and a thickness of 40 mils for the walls defining the chambers 32 and 34 is adequate in the illustrated operational embodiment. The wells of the test strip, including the first and second chambers 32 and 34, respectively, are covered with a thin film or membrane 64 after manufacture, shown in Figures 7, 11, 12, to seal all of the wells and reaction vessel 10. The membrane (such as PET, commonly known as MYLAR, or aluminum foil with a more-prime polyethylene/polypropylene mix adhesive) is removed from Figures 5, 8 and 10 in order to illustrate the structures in the test strip 19.

The bottom of the first chamber 32 is capped by a cap 60 that is ultrasonically welded to the bottom surface 68 of the walls defining the first chamber. The cap 60 is shown greatly enlarged in Figures 16-18 and discussed below. The cap 60 provides a fluid passage from the base of the first chamber 32 to the base of an intermediary fluid passage 70 connecting the first chamber 32 to the second chamber 34. A plunger 56 with a chisel-like tip is positioned in the intermediary fluid passage 70. The chisel tip of the plunger 56 breaks a membrane or seal 72 (Figure 9) in the fluid passage (flushed molded in the fluid passage during molding) when the plunger 56 is depressed from above. This allows fluid to migrate from the first chamber 32 into the fluid passage 70, up along the side of the plunger 56 and into a second channel 74 (Figures 8 and 10) communicating with a enzyme pellet chamber 76 that contains the enzyme pellet (not shown). The fluid sample dissolves the enzyme pellet as it travels through the enzyme pellet chamber 76 into the second or amplification chamber 34 (see Figure 8).



A vacuum port 80 (Figure 8) is provided in fluid communication with the second chamber 34. A Porex polyethylene filter (not shown) is positioned within the vacuum port 80. Vacuum is used to effectuate the transfer of the fluid sample from the first chamber 32 to the second chamber 34 after the plunger 56 has been moved to the lower position to break the seal 72. A vacuum implement containing a vacuum probe or tube (see e.g., Figure 33) is inserted into the vacuum port 80 in a manner such that a seal is formed in the top surface 82 of the strip adjacent the vacuum port 80. Vacuum is drawn in the vacuum tube. The pressure difference resulting from ambient pressure in the first chamber 32 and a vacuum in the second chamber 34 draws fluid up the intermediate chamber or fluid passage 70 and into the channel 74 and pellet chamber 76 and into the second chamber 34.

Figure 13 is an isolated perspective view of the plunger 56 of Figure 12. Figure 14 is another perspective view of the plunger 56, shown from below. Figure 15 is an elevational view of the plunger 15. Referring to Figures 13-15, the plunger includes a cylindrically-shaped body 90 having a chisel 92 at the lower end thereof and a head portion 94. The head portion 94 includes a circular ring 96 with voids 98 formed therein to promote the drawing of a vacuum in the intermediate chamber 70 (Figures 8-12) in which the plunger is installed. The head 94 has downwardly depending feet 100 that seat on a rim 102 (Figure 11) inside the intermediate chamber 70 when the plunger 65 has been depressed to its lowermost position, as shown in Figure 12. The chisel 92 has a tip 104 that breaks through the seal or membrane 72 obstructing the passage of fluid up the intermediate channel 70. The seal 72 is best showing Figures 9, 11 and 12. Figure 12 shows the placement of the chisel 92 just above the seal 72 as it would be while the heating to 95 degrees C in the first chamber 32 is occurring and during the cool-down period.

As shown in Figure 14, the plunger has a V-shaped groove 106 in the side of the plunger body 90 that provides a channel for fluid to rise up the length of the cylindrical body 90 of the plunger to the elevation of channel 74 (Figure 10) connecting the intermediate chamber 70 with the enzyme pellet chamber 76.

Figure 16 is a perspective view of the top surface of the cap 60 that covers the base of the first chamber of the reaction vessel of Figures 8 and 9, shown greatly enlarged. Figure 17 is a cross-section of the cap 60 of Figure 16. Figure 18 is a perspective view of the base of cap 60. Referring to these figures, in conjunction with Figures 6 and 9, it will be seen from Figure 8 that without the cap 60 there is no base to the first chamber 32 and no fluid passage between the first chamber 32 and the intermediary chamber 70. The cap 60 provides the base of the first chamber 32 and the passage between the first chamber 32 and the intermediate chamber 70. The cap 60 includes a shallow tray 110 positioned to form a

base of the first chamber 32. The tray 110 slopes downwardly to a small passage 112 linking the shallow tray 110 to a circularly shaped reservoir 114 that is in vertical alignment with the circular wall 116 of the intermediate chamber (see Figure 9). The semirectangular and semicircular rim 118 of the cap 60 is ultrasonically bonded to the bottom portions 68 and 116 of the first and intermediate chambers, respectively, as shown in Figure 6. In the installed condition, when the fluid sample has been introduced into the first chamber 32, the fluid will pass into the channel 112 and reservoir 114, immediately below the seal 72 in the intermediate chamber (see Figure 9). Thus, when the seal 72 is broken by the plunger 56 and vacuum is drawn from the vacuum port 80 of Figure 8, the solution of the fluid sample and reagent from the first chamber 32 will be drawn up the side of the plunger 56 and into the enzyme pellet chamber 76, dissolving the pellet, and into second chamber 34 where the amplification reaction takes place.

Referring to Figure 5, after the amplification reaction has occurred in the second chamber 34 at the proper temperature, the SPR (not shown) is lowered into the second chamber 34 and a portion of the amplified sample is withdrawn into the SPR. The SPR and test strip are moved relative to each other such that the SPR is positioned above the adjacent probe well 36, whereupon it is lowered into the probe well 36. The rest of the analytical processes with the SPR and test strip are conventional and well known in the art. For example, the process may be implemented in the manner performed by the VIDAS instrument of the applicants' assignee.

Figure 19 is a perspective view of a stand-alone disposable dual chamber reaction vessel 10 that is designed to snap into the test strip 19 of the type shown in Figure 5 in the manner suggested in Figure 4. Figure 20 is a perspective view of the stand-alone disposable dual chamber reaction vessel of Figure 19 shown upside down, with a lower cap constructed as shown in Figure 16-18 to cover the base of the first chamber 32 and intermediate chamber 70 removed. A thin film or foil type membrane is applied to the top surface of the reaction vessel 10, in a manner to cover the first chamber 32, the intermediate chamber 34, enzyme pellet chamber 76, second chamber 34 and vacuum port 80. The film is not shown in Figure 19 in order to better illustrate the structures of the reaction vessel 10. Further, a plunger for the intermediate chamber 70 is also not shown. Once the stand-alone disposable reaction vessel of Figures 19 and 20 has been installed into the test strip, the operation of the embodiment of Figures 19 and 20 is exactly as described above.

To accommodate the vessel of Figures 19 and 20 into the test strip 19 of Figures 5 and 6, the test strip 19 is modified by providing an aperture in the left hand end 24 of the test strip adjacent to the probe well 36, and providing suitable rail structures to allow a pair of flanges 120 on the periphery of the unit 10 to snap into the test strip 19. Of course, it will be understood that after mold-

ing of the reaction vessel of Figure 19, the nucleic acid and amplification reagent will be added to the first chamber 32, and the enzyme pellet is added to the enzyme pellet chamber 76. Then, the film covering the entire top surface of the vessel 10 will be applied to seal the chambers. The device is then ready for use as described herein.

Figure 21 is perspective view of yet another alternative construction of the disposable dual chamber reaction vessel 10 of Figure 19 that can be molded into the test strip or made as a separate unit to snap into a test strip 19 as described above. The vessel 10 has a first chamber 32 and a second chamber 34 and an intermediate chamber 70 linking the two chambers 32 and 34 together. The base of the first chamber 32 has a hole that is plugged with a cap 60 that is ultrasonically welded to the base of the housing 130. The cap 60 is spaced slightly from the bottom surface of a wall 132 forming the side of the first chamber 32, thereby defining a small passage 134 for fluid to flow out of the first chamber into the intermediate chamber 70. Amplification reagents 16 for the denaturation step are loaded into the base of the chamber 32 of the reaction vessel 10, as shown in Figure 25. An enzyme pellet 18 is loaded into the secondary chamber 34.

An elastomeric thimble-shaped valve element 140 having helical rib features 142, shown isolated in Figure 24, is positioned in the intermediate chamber 70. Figure 22 is a cross-sectional view of the embodiment of Figure 21, showing the thimble valve 140 in the intermediate chamber 70. A filter 144 is positioned above the top of the thimble valve 144. In its relaxed state, a lower circumferential rib 148 on the thimble valve 140 and the exterior surfaces of the helical rib feature 142 on the side walls of the thimble valve 140 make contact with the wall of the intermediate chamber 70, sealing off the chamber 70 and preventing fluid from passing from the gap 134 separating the cap 60 from the wall 132, up the intermediate chamber 70 and into the secondary chamber 34.

The resilient thimble valve 140 is deformable such that the lower circumferential rib 148 may be moved away from the wall of the intermediate chamber 70. This is achieved by inserting an element 152 into the interior of the thimble valve 140 and pressing on the wall portion 149 of the valve 140 to stretch and deform the end wall and adjacent shoulder of the thimble valve. Figure 23 is a cross-sectional view of the embodiment of Figure 21 showing the action of the helical thimble valve 140 being deformed by a vacuum plunger 152 that is inserted into the interior of the thimble valve 140. The end of the vacuum plunger presses against the wall 149, as shown in Figure 23, pulling the lower circumferential rib away from the wall of the intermediate chamber 70. The helical rib feature 142 stays in contact with the cylindrical wall of the chamber 70. At the same time, vacuum is drawn through an aperture in the side of the vacuum plunger 152 to pull air out of the secondary chamber 34 and through the filter 144 into the vacuum plunger 152.

This vacuum action draws fluid out of the base of the first chamber 32, and up vertically in a helical path along the helical port defined between the helical rib feature 142 and the wall of the intermediate chamber 70. Substantially all of the patient sample/reagent solution in the first well 32 is removed in accordance with this embodiment. The solution passes from the upper end of the helical feature 142 into a gap 150 connecting the intermediate chamber 70 with the second chamber 34. This is illustrated best in Figures 23 and 25.

The embodiment of Figures 21-23 has the advantage that the opening of the thimble valve 140 tends to cause any oil in the amplification reagent mix in the first chamber that may find its way to the base of the intermediate chamber 70 to be blown back toward the first chamber, acting in the manner of a common plunger, and allow the fluid sample and reagent solution to take its place. Where the amplification reagent contains an oil such as a silicone oil, it is important that the oil is not the first substance to migrate into the second chamber, as this can cause the oil to coat the enzyme pellet in the second chamber, which can interfere with the amplification reaction in the second chamber 34. Thus, preferably the thimble valve 140 is designed such that when the wall 149 of the thimble valve 140 is activated by the vacuum probe 152, any oil that may lie at the base of the intermediate chamber 70 is initially forced back into the first chamber 32. Once the lower rib 148 of the thimble valve 140 is moved away from the wall of the intermediate chamber 70, the drawing of the vacuum in the second chamber allows the fluid sample/reagent solution to be drawn into the second chamber as described above.

Figure 26 is a perspective view of yet another embodiment of the disposable reaction vessel 150 in accordance with the invention. The reaction vessel 150 is designed to snap into the test strip 19 of Figure 8 in the manner suggested in Figure 4 and described above. Figure 27 is a cross-section of the embodiment of Figure 26. Referring to Figures 26 and 27, the disposable reaction vessel 150 comprises a unitary housing 152 that defines a first chamber or amplification well 154 which has loaded in it an amplification pellet or dried reagent mix 16 for the denaturation step in the TMA process. The amplification well 154 is separated from a second chamber 156 by a heat and moisture isolation barrier 158. The second chamber contains an enzyme plunger or carrier 160 for containing an enzyme pellet 18 for introduction into the amplification well 154 after the fluid sample has been introduced into the amplification well 154 and the denaturation process has been completed. The enzyme plunger 160 has a recessed surface 162 for receiving an implement through the opening at the top of the chamber 156. A foil layer 164 is applied to the top surface of the reaction vessel 150 as shown.

Figure 28 is a cross-section of a test strip 19 incorporating the embodiment of Figure 26. The reaction vessel 150 can be manufactured as a stand-alone disposable unit, as suggested in Figures 26 or 27, and snapped

into place in a test strip as shown in Figure 28, or the test strip of Figure 28 may be manufactured with the amplification well of Figure 31 as an integral part of the test strip 19 itself. In the preferred embodiment, the unit 150 is manufactured as an integral part of the test strip. The test strip 19 has a sliding cover 164 positioned at the end of the test strip 19 comprising a gripping surface 166 and a plastic label 168 carried by first and second mounting structures 170.

Figures 29A-29C show the use of the test strip 19 with the disposable reaction vessel of Figure 28. In the first step, the sliding cover 164 is pulled back and a pipette 172 is inserted through the foil layer 164 to deposit the fluid sample 176 into the amplification well 154. The pipette 172 is removed and the cover 164 is slid back into place over the amplification well 154 into the position shown in Figure 29B. The amplification well 154 is heated to 95 degrees C to subject the fluid sample 176 to denaturation with the aid of the amplification reagent pellet 16. The second chamber 156 containing the enzyme pellet 18 is not subject to the 95 degree C heating. After the amplification well has cooled down to 42 degrees C, an implement 180 is inserted into the second chamber containing the enzyme carrier 160 and enzyme pellet 18 and placed into contact with the enzyme carrier 160. The implement 180 is moved further in to force the carrier 160 through the heat and moisture isolation barrier 158, thereby adding the enzyme pellet 18 to the amplification well 154. The enzyme carrier 160 blocks the chamber as shown in Figure 29 C, preventing contamination of the amplification well 154. A cover (not shown) could be slid over the entrance of the second chamber or channel if desired. The amplification well 154 is then maintained at a temperature of 42 degrees C for roughly one hour for the amplification process to proceed. After the amplification process is complete, a reagent SPR having at least one reaction zone is inserted through a membrane 168 or label as shown in Figure 29 C, and a portion of the amplified solution is withdrawn into the SPR. The rest of the process proceeds in known fashion.

Figure 30 is a schematic representation of yet another embodiment of a dual chamber disposable reaction vessel 10. The fluid sample is loaded into the first chamber 32 and denaturation and primer annealing steps are performed in the first chamber 32, with the aid of an amplification mix reagent loaded into the first chamber. After the first chamber has cooled to 42 degrees C, a piston mechanism 184 is applied to the first chamber 184 to increase the fluid pressure in the first reaction chamber to break a seal 186 in a fluid channel 188 connecting the first chamber 32 to the second chamber 34. The fluid sample is forced from the first chamber 32 into the second chamber 34. The second chamber is loaded with the enzyme pellet 18. The amplification reaction takes place in the second chamber 34 at a temperature of 42 degrees C. The piston 184 may be incorporated as a cap structure to the reaction

vessel 10 and which is depressed by a SPR, as shown, or a separate piston could be used to force the fluid from the first chamber 32 into the second chamber 34.

Figure 31 is a perspective view of a stand-alone amplification reaction processing system 200 for the test strips 19 (see, e.g., Figures 3 and 5) having the dual chamber reaction vessels in accordance with a presently preferred form of the invention. The system 200 consists of two identical amplification stations 202 and 204, a power supply module 206, a control circuitry module 208, a vacuum tank 210 and connectors 212 for the power supply module 206. The tank 210 has hoses 320 and 324 for providing vacuum to amplification stations 202 and 204 and ultimately to a plurality of vacuum probes (one per strip) in the manner described above for facilitating transfer of fluid from the first chamber to the second chamber. The vacuum subsystem is described below in conjunction with Figure 39.

The amplification stations 202 and 204 each have a tray for receiving at least one of the strips 19 of Figure 5 (in the illustrated embodiment up to 6 strips) and associated temperature control, vacuum and valve activation subsystems for heating the reaction wells of the strip to the proper temperatures, effectuating a transferring of fluid from the first chamber in the dual chamber reaction wells to the second chamber, and activating a valve such as a thimble valve in the embodiment of Figure 22 to open the fluid channel to allow the fluid to flow between the two chambers.

The stations 202 and 204 are designed as stand alone amplification stations for performing the amplification reaction in an automated manner after the patient or clinical sample has been added to the first chamber of the dual chamber reaction vessel described above. The processing of the strips after the reaction is completed with a SPR takes place in a separate machine, such as the commercially available VIDAS instrument. Specifically, after the strips have been placed in the stations 202 and 204 and the reaction run in the stations, the strips are removed from the stations 202 and 204 and placed into a VIDAS instrument for subsequent processing and analysis in known fashion.

The entire system 200 is under microprocessor control by an amplification system interface board (not shown in Figure 31). The control system is shown in block diagram form in Figure 38 and will be described later.

Referring now to Figure 32, one of the amplification stations 202 is shown in a perspective view. The other amplification station is of identical design and construction. Figure 33 is a perspective view of the front of the station 202 of Figure 31.

Referring to these figures, the station includes a vacuum probe slide motor 222 and vacuum probes slide cam wheel 246 that operate to slide a set of vacuum probes 244 (shown in Figure 33) for the thimble valves of Figure 21 up and down relative to a vacuum probes slide 246 to open the thimble valves (reference 140 in

the embodiment of Figures 21-23) and apply vacuum so as to draw the fluid from the first chamber of the reaction vessel 10 (e.g., Figure 21) to the second chamber. The vacuum probes 244 reciprocate within annular recesses provided in the vacuum probes slide 246. The vacuum probes 244 are positioned in registry with the intermediate chamber 70 in the embodiment of Figure 22, or in registry with the vacuum port 80 in the embodiment of Figure 11.

For an embodiment in which the strips are constructed in the manner of Figures 5-12, the vacuum probe 244 would incorporate a suitable pin structure (not shown) immediately adjacent the shaft of the vacuum probe 244 that would operate the plunger 56 of Figure 12 to open the intermediate chamber 70 when the vacuum probe 244 is lowered onto the vacuum port. Obviously, proper registry of the pin structure and vacuum probe 244 with corresponding structure in the test strip as installed on the tray needs to be observed.

The station includes side walls 228 and 230 that provide a frame for the station 202. Tray controller board 229 is mounted between the side walls 228 and 230. The electronics module for the station 202 is installed on the tray controller board 229.

A set of tray thermal insulation covers 220 are part of a thermal subsystem and are provided to envelop a tray 240 (Figure 33) that receives one or more of the test strips. The insulation covers 220 help maintain the temperature of the tray 240 at the proper temperatures. The thermal subsystem also includes a 42 degree C Peltier heat sink 242, a portion of which is positioned adjacent to the second chamber in the dual chamber reaction vessel in the test strip to maintain that chamber at the proper temperature for the enzymatic amplification reaction. A 95 degree C heat sink 250 is provided for the front of the tray 240 for maintaining the first chamber of the reaction well in the test strip at the denaturation temperature.

Figure 34 is another perspective view of the module of Figure 33, showing the 95 degree C heat sink 250 and a set of fins 252 dissipating heat. Note that the 95 degree C heat sink 250 is positioned to the front of and slightly below the tray 240. The 42 degree C heat sink 242 is positioned behind the heat sink 250.

Figure 35 is a detailed perspective view of a portion of the tray 240 that holds the test strips (not shown) as seen from above. The tray 240 includes a front portion having a base 254, and a plurality of discontinuous raised parallel ridge structures 256 with recessed slots 258 for receiving the test strips. The base of the front 254 of the tray 240 is in contact with the 95 degree C heat sink 250. The side walls of the parallel raised ridges 256 at positions 256A and 256B are placed as close as possible to the first and second chambers of the reaction vessel 10 of Figure 1 so as to reduce thermal resistance. The base of the rear of the tray 240 is in contact with a 42 degree C Peltier heat sink, as best seen in Figure 34. The portion 256B of the raised ridge for the rear of the

tray is physically isolated from portion 256A for the front of the tray, and portion 256B is in contact with the 42 degree C heat sink so as to keep the second chamber of the reaction vessel in the test strip at the proper temperature.

Still referring to Figure 35, each of the vacuum probes 244 include a rubber gasket 260. When the vacuum probes 244 are lowered by the vacuum probe motor 222 (Figure 32) the gaskets 260 are positioned on the film covering the upper surface of the test strip surrounding the vacuum port in the dual chamber reaction vessel so as to make a tight seal and permit vacuum to be drawn on the second chamber.

Figure 36 is an isolated perspective view of the test strip holder or tray 240 of Figure 35, showing two test strips 19 in accordance with Figure 5 installed in the tray 240. The tray 240 has a plurality of lanes or slots 241 receiving up to 6 test strips 19 for simultaneous processing. Figure 36 shows the heat sinks 242 and 250 for maintaining the respective portions of the tray 240 and ridges 256 at the proper temperature.

Figure 37 is a detailed perspective view of the test strip holder or tray 240 as seen from below. The 95 degree C Peltier heat sink which would be below front portion 254 has been removed in order to better illustrate the rear heat sink 242 beneath the rear portion of the tray 240.

Figure 38 is a block diagram of the electronics and control system of the amplification processing system of Figure 31. The control system is divided into two boards 310 and 311, section A 310 at the top of the diagram devoted to amplification module or station 202 and the other board 311 (section B) devoted to the other module 204. The two boards 310 and 311 are identical and only the top section 310 will be discussed. The two boards 310 and 311 are connected to an amplification station interface board 300.

The interface board 300 communicates with a stand alone personal computer 304 via a high speed data bus 302. The personal computer 304 is a conventional IBM compatible computer with hard disk drive, video monitor, etc. In a preferred embodiment, the stations 202 and 204 are under control by the interface board 300.

The board 310 for station 202 controls the front tray 240 which is maintained at a temperature of 95 degrees C by two Peltier heat sink modules, a pair of fans and a temperature sensor incorporated into the front portion 254 of the tray 240, all of which are conventional. The back of the tray is maintained at a temperature of 42 degrees C by two Peltier modules and a temperature sensor. The movement of the vacuum probes 244 is controlled by the probes motor 222. Position sensors are provided to provide input signals to the tray controller board as to the position of the vacuum probes 244. The tray controller board 310 includes a set of drivers 312 for the active and passive components of the system which receive data from the temperature and position sensors and issue commands to the active compo-

nents, i.e., motors, fans, Peltier modules, etc. The drivers are responsive to commands from the amplification interface board 300. The interface board also issues commands to the vacuum pump for the vacuum subsystem, as shown.

Figure 39 is a diagram of the vacuum subsystem 320 for the amplification processing stations 202 and 204 of Figure 31. The subsystem includes a 1 liter reinforced plastic vacuum tank 210 which is connected via an inlet line 322 to a vacuum pump 323 for generating a vacuum in the tank 210. A vacuum supply line 324 is provided for providing vacuum to a pair of pinch solenoid valves 224 (see Figure 32) via supply lines 324A and 324B. These vacuum supply lines 324A and 324B supply vacuum to a manifold 226 distributing the vacuum to the vacuum probes 244. Note the pointed tips 245 of the vacuum probes 244 for piercing the film or membrane 64 (Figure 11) covering the strip 19. The vacuum system 320 also includes a differential pressure transducer 321 for monitoring the presence of vacuum in the tank 210. The transducer 321 supplies pressure signals to the interface board 300 of Figure 38.

Figure 40 is a representative graph of the thermal cycle profile of the station of Figure 31. As indicated in line 400, after an initial ramp up 402 in the temperature lasting less than a minute, a first temperature T1 is reached (e.g., a denaturation temperature) which is maintained for a predetermined time period, such as 5-10 minutes, at which time a reaction occurs in the first chamber of the reaction vessel. Thereafter, a ramp down of temperature as indicated at 404 occurs and the temperature of the reaction solution in the first chamber of the reaction vessel 10 cools to temperature T2. After a designated amount of time after cooling to temperature T2, e.g., 42 degrees C, a fluid transfer occurs in which the solution in the first chamber is conveyed to the second chamber. Temperature T2 is maintained for an appropriate amount of time for the reaction of interest, such as one hour. At time 406, the temperature is raised rapidly to a temperature T3 of  $\geq 65$  degrees C to stop the amplification reaction. For a TMA reaction, it is important that the ramp up time from time 406 to time 408 is brief, that is, less than 2 minutes and preferably less than one minute. Preferably, all the ramp up and ramp down of temperatures occur in less than a minute.

Referring now to Figure 41, an alternative and preferred construction for the dual chamber reaction vessel that is suitable for use with the reaction processing station of FIGs. 30-39 and the test strip described previously is illustrated. This embodiment provides a valve means for controlling a connecting conduit linking the first and second chambers together. The valve means was particularly simple to put into effect, both with respect to the construction or design of the reaction vessel and with respect to the external means required for controlling or activating these components.

The valve means includes three components and associated features. First, a connecting conduit is pro-

vided which is flexible, that is to say having an internal cross-section of flow which can be reduced simply by the application of external pressure, or having a wall which can yield (i.e., deflect inwardly), again by the application of this external pressure. Second, a sealing piece or ball element is disposed within the conduit. This seal piece provides a hermetic seal within the connecting conduit. The seal piece is held in the conduit by the wall of the conduit being pressed against the external surface of the seal piece. Thirdly, the conduit and seal piece are adapted to work together with an external device for constricting the conduit element externally, and set up or positioned in relation to this external device to create a primary or interstitial passage within this conduit piece at the point where the seal piece is located.

Referring now to Figures 41 to 43, a dual chamber reaction vessel 10 in accordance with this embodiment includes a molded body 512 of plastic material. The two flat faces at the front and rear of the body are coated with two films of material (513 and 514 respectively) which seal off the first and second reaction chambers and passages created in the body 512 by the molding process.

Figures 41 and 42 clearly show how the two reaction chambers 502 and 503 are formed, mainly in the body section 512, with one chamber 502 being cylindrical and tapered in shape and the other 503 having a quadrangular cross-section. These two chambers are joined together by a connecting flexible conduit 504 similar to a siphon. One end of the conduit 504 is in communication via a front orifice 510 to the lower part of the chamber 502. The other end of the conduit 504 has a rear orifice 511 set at the top of the other chamber 503, and passing via a vertical conduit portion 505 which is described in further detail below.

A means to control, in particular to open, the connection conduit 504 described above is provided in the conduit portion 505. In particular, an external device 508 is provided for constricting the conduit portion 505. The external device 508 is inserted into the reaction vessel 10 from the side to which the equipment or control system is connected to the conduit portion 505, for example from above the test strip when the reaction vessel is positioned in a test strip and installed in the processing station of FIGs. 31-39.

As shown in FIGs. 41-44, in a first embodiment, the conduit portion 505 is flexible, meaning that its internal cross-section can be reduced by applying an external pressure, such as pressure applied peripherally or centripetally. As with the body 512, this conduit piece 505 is made from plastic material, such as low density polyethylene for example.

A substantially rigid seal piece 506, consisting of a ball of glass or metal, is held in the interior 505a of the conduit portion 505. The seal piece 506 is held in place solely by the force of wall 507 of the conduit portion being pressed against the external surface of the seal piece 506. The seal piece 506 and the internal cross-

section of the inside of the conduit portion 505a are both arranged so that the position for the seal piece 506 ensures that the seal piece provides a tight seal on the inside of the conduit portion 505a.

The conduit portion 505 consists of two parts. The first part 505b has a relatively narrow internal cross-section in which the seal piece 506 is held by the pressing action. The second part 505c has a relatively wide internal cross-section in which the seal piece 506 cannot be held by the pressing action and therefore falls to the bottom of the connecting conduit 504.

As stated previously, an external device 508 is provided on the automatic analysis apparatus side (i.e., above the dual chamber reaction vessel) to constrict the conduit portion 505. This external device is represented schematically in Figures 43 and 44 by two arms (581 and 582) fitted with pinch bars (581a and 582a respectively). Openings 521 and 522 are provided in the body 512 on either side of the conduit portion 505 to allow the two arms 581 and 582 to move freely (upwards and downwards, for example) and into a position for cooperating with the ball or seal piece 506. For example, and with reference to FIG. 33, each of the vacuum probe tools 244 may incorporate arm elements 581 and 582 which cooperate with the seal piece 506 to open the conduit 505 when they (tools 244) are lowered down onto the test strip.

As shown in Figure 44, the external constriction device 508 is positioned to move along the conduit portion 505 and push the seal piece 506 from the first part of the conduit portion 505b to the second part 505c without coming into contact with it. This allows the seal piece 506 to fall to the bottom of the conduit portion and free or open the passage in the conduit piece.

Two external stops 505d (Figure 41) are provided on the outside of the conduit portion to stop movement, for example downward movement, of the arms 81 and 82.

Referring now to Figure 45, in a second variation of the embodiment of Figure 41, the wall 507 of the conduit device 507 can yield, again by the application of external pressure, for example pressure applied peripherally or centripetally, when the relatively hard seal piece 506 comes into contact with it. In this case, the constricting device 508 is set up so that when it is in its lowered position, it makes an impression of the seal piece 506 in the wall 507 to create a lasting internal imprint 509. When the external constricting device 508 releases this pressure, an interstitial passage is created after the constriction device 508 has acted between the seal piece and the wall 507. This interstitial passage enables or releases flow through the connecting conduit 504. The dotted line to the left of Figure 45 shows the ball 506 in the position it is held in conduit 505, with the solid line at the right of the illustration showing the imprint made by the action of the constricting device 508.

Another representative example of how the dual chamber reactions vessels of this disclosure may be

loaded with fluid sample and of how the fluid samples may be transferred from one chamber to another will be described in conjunction with Figure 46 and 47A-47E.

As shown on Figure 46, a dual chamber reaction vessel 600 comprising a body 612 made for example from molded plastic material. The vessel 600 includes a first chamber 602, made from plastic material, in communication with the outside via a conduit 604, with the closure and/or opening of this conduit controlled by a system, such as a valve, which is represented schematically by reference number 606. On the other side of the control system 606, this first conduit is in communication with an angled sampling conduit 608, which is described in further detail below. The vessel also includes a second chamber 603 in communication with the first chamber 602 only, via a second connecting conduit 605, which also has closing and/or opening operations controlled by a system, such as a valve, which is represented by the general reference number 607. The valve 607 and conduit 605 may, for example, take the form of the conduit and ball valve described previously, the elastomeric thimble valve and conduit described earlier, or the spike structure that is operated to pierce a membrane and described above.

The component of the type illustrated in Figure 46 is generally operated within a gaseous external environment, at a reference pressure, hereinafter termed high pressure, for example atmospheric pressure.

Further, the first and second chambers are loaded with reagent and enzymes in the manner described previously at the time of manufacture.

As an example, a first chemical or biochemical reaction takes place in the first chamber 602, causing this chamber to contain a first reagent, and the reagent product obtained in chamber 602 is subjected to a further reaction in chamber 603, causing chamber 603 to contain a reagent or product which is different from the reagent originally contained in chamber 602.

A process is illustrated in Figures 47A-47F whereby a liquid sample 611 contained in an external container, a test tube 610 for example, is transferred into the first chamber 602 and then into the second chamber 603. The second chamber 602 is originally under high pressure, with the second conduit 605 being closed, and chambers 602 and 603 are isolated from each other. With the first conduit 604 being open, the first chamber 602 is in communication with the external environment and is therefore under high pressure HP (see Fig. 47A).

The first chamber 602 is brought down to a reduced pressure by the first conduit 604, i.e., a pressure being lower than the pressure termed low pressure which is described in further detail below; this is achieved by means of an arrangement such as connecting the first conduit 604 to an evacuation device or pump 609 (see Fig. 47B). The first conduit 604 is then closed.

The free end of the angled tube 608 is immersed in the liquid 611 to be transferred contained in container 610. The first conduit 604 is in communication with the

liquid at an immersed level via this angled tube 608, with the liquid being located in the gaseous external environment and hence subjected to high pressure. The first conduit is then opened, causing the liquid to be transferred into the first chamber 602 via the first conduit 604 (see Figure 47C). Finally, the pressure in the first chamber 602 becomes established at a value termed reduced pressure (RP) which is greater than the pressure termed low pressure mentioned above, although remaining lower than the pressure termed as high pressure.

The first conduit 604 is closed to produce the situation shown in Figure 47D. The second conduit 605 is closed and the two chambers 602 and 603 are isolated from each other, with the second chamber 603 being at high pressure with the first conduit 604 closed, and the second chamber 602 being isolated from the outside and partially filled with the liquid previously transferred, whilst being at reduced pressure.

The second conduit 605 is opened (i.e., by opening the valve 607), causing the pressure in the two chambers 602 and 603 to become balanced at a pressure termed intermediate pressure (IP) which is between the high and reduced pressure values (see Figure 47E).

The first conduit 604 is then opened, causing the first chamber 602 to be in communication with the external high pressure environment, and the liquid is transferred from the first chamber 602 to the second chamber 603 via the second conduit 605 (see Figure 47F). The pressure in the two chambers finally reaches the high pressure value. The first conduit 604 can be sealed permanently when the entire process has been completed. The reaction can then proceed in chamber 603. Of course, chambers 602 and 603 may be maintained at separate temperatures in accordance with the principles of the invention set forth above.

While presently preferred embodiments of the invention have been described herein, persons of skill in the art will appreciate that various modifications and changes may be made without departure from the true scope and spirit of the invention. For example, the novel reaction vessels and test strips can be used in other reactions besides isothermal amplification reactions such as TMA. The invention is believed to be suitable for many isothermal reactions, other enzymatic reactions, and reactions requiring differential heating and containment. For example, the reference to "denaturation and cooling", while specifically applicable to the TMA reaction, can be considered only one possible species of a heat differential step. Further, the spatial and temperature isolation of the amplification enzyme in the second chamber is considered one example of spatial isolation of a heat labile reagent. The invention is fully capable of being used in other types of reactions besides TMA reactions. This true scope and spirit is defined by the claims, to be interpreted in light of the foregoing.

## Claims

1. A dual chamber reaction vessel for a nucleic acid amplification reaction, characterised in that it comprises:
  - a first chamber for receiving a fluid sample, the said first chamber being loaded with one or more amplification reagents;
  - a second chamber physically isolated from the said first chamber;
  - one or more enzymes for the said reaction either in the said second chamber or in fluid communication with the said second chamber;
  - and
  - a fluid channel connecting the said first chamber to the said second chamber.
2. A vessel as claimed in claim 1 wherein it further comprises a means for selectively opening the said fluid channel to allow a fluid to pass from the said first chamber into the said second chamber.
3. A vessel as claimed in claim 1 or claim 2 wherein the said means for selectively opening the said fluid channel comprises a breakable seal, preferably the said means for selectively opening the said fluid channel comprising a means for creating fluid pressure in the said sample fluid so as to break the said seal and enable fluid to flow from the said first chamber through the said fluid channel into the said second chamber, or the said means for selectively opening the said fluid channel comprising a plunger reciprocable within the said vessel from a first position to a second position, the said plunger in the said second position being operable to cut the said seal and thereby open the said fluid channel; or the said means for selectively opening the said fluid channel comprising a valve, preferably a thimble valve.
4. A vessel as claimed in any of claims 1 to 3 wherein it further comprises a vacuum port in fluid communication with the said second chamber, such that the application of vacuum to the said vacuum port draws the said fluid sample from the said first chamber into the said second chamber.
5. A dual chamber reaction vessel characterised in that it comprises:
  - a first chamber and a second chamber joined together via a connecting conduit;
  - and
  - a valve means for opening the said conduit comprising:

(a) a flexible conduit portion linking the said

first and second chambers having a wall portion:

(b) a substantially rigid seal piece disposed within the said flexible conduit: the said seal piece providing a tight seal within the said conduit portion and being held in the conduit by the said wall of the conduit portion pressed against the said seal piece:

and

(c) an external device for constricting the said conduit portion: the said conduit piece cooperating with the said external device for constricting the said conduit portion, the said conduit portion being positioned in relation to the said external device such that relative motion between the said conduit portion and the said external device causes the said constricting device to act on the said seal piece to open the said conduit portion and create a passage within the said conduit portion at the point where the said seal piece is located.

6. A vessel as claimed in claim 5 wherein the said seal piece comprises a ball.

7. A vessel as claimed in claim 5 or claim 6 wherein the said conduit portion, which is preferably made from a flexible plastic material, further comprises an internal section which may be reduced by the application of an external pressure and consists of a first portion having a relatively narrow internal cross-section in which the seal piece is held by the wall of the said conduit portion, and a second portion with a relatively wide cross-section in which the said seal piece cannot be held by the said wall, the said first and second portions being oriented such that the said external constriction device may be moved along the said conduit portion to push the said seal piece from the said first portion to the said second portion, and, optionally, further comprising at least one external stop incorporated on the outside of the conduit portion to halt the movement of the constriction device into the said dual chamber reaction vessel.

8. A vessel as claimed in any of claims 5 to 7 wherein the said conduit piece further comprises a relatively yielding wall and the external constriction device is operative to make an impression of the shape of the outside of the seal piece in the said wall to create an imprint on the inside of the said wall to allow an interstitial flow between the said seal piece and the said wall after action of the constriction device.

9. A vessel as claimed in any of claims 5 to 8 wherein the said first and second reaction vessel and the said conduit portion are made from a single mould-

ing of plastic material.

10. A vessel as claimed in any of claims 5 to 9 wherein the said external constriction device comprises a pair of arms, the said arms cooperating with the said seal piece to move the said seal piece from a first location in the said conduit portion to a second location in the said conduit portion when the said pair of arms are moved relative to the said dual chamber reaction vessel.

11. A two-piece disposable reaction vessel characterised in that it comprises:

a first piece comprising a first chamber containing an amplification reagent:

and

a second piece comprising a second chamber containing an amplification enzyme:

the said first and second pieces being constructed so as to permit the said first and second pieces to be securely joined into a dual chamber reaction vessel such that a fluid sample in the said first chamber may pass into the said second chamber.

12. A reaction vessel for a nucleic acid amplification reaction characterised in that it comprises:

an amplification well;

a channel separated from the said amplification well by a heat and moisture barrier;

a carrier in the said channel carrying an amplification enzyme;

the said carrier being moveable within the said channel such that the said carrier may be moved through the said heat and moisture barrier to deliver the said enzyme to the said amplification well.

13. A nucleic acid amplification station for a test strip comprising a dual chamber reaction vessel comprising a first chamber and a second chamber, characterised in that the station comprises:

a tray for at least one test strip, the said tray comprising a first portion and a second portion positioned adjacent to the said first and second chambers of the said dual chamber reaction vessel, respectively;

a temperature control subsystem for the said tray maintaining the said first and second portions of the said tray at first and second different amplification reaction temperatures so as to maintain the said first and second chambers at the said first and second amplification reaction temperatures, respectively;

a fluid conduit opening mechanism for opening



a fluid conduit in the said dual chamber reaction vessel to establish fluid communication between the said first chamber and the said second chamber;

and, optionally,

a vacuum subsystem comprising a vacuum probe, the said test strip and vacuum probe being mutually reciprocable and the said vacuum probe cooperating with reaction wells in the said test strip for transferring a fluid sample from the said first chamber to the said second chamber via the said fluid conduit.

14. A station as claimed in claim 13 wherein the said fluid conduit opening mechanism comprises a pin for the said test strip reciprocable with the said vacuum probe relative to the said test strip.

15. A station as claimed in claim 13 or claim 14 wherein the said vacuum probe comprises a tip portion and the said fluid conduit opening mechanism comprises the said tip portion of the said vacuum probe.

16. A station as claimed in any of claims 13 to 15 wherein the said temperature control subsystem further comprises a first heat sink for heating the said first portion of the said test strip to the said first temperature and a second heat sink, isolated from the said first heat sink, for maintaining the said second portion of the said test strip at the said second temperature.

17. A station as claimed in any of claims 13 to 16 wherein the said tray comprises a base and a plurality of raised ridges defining slots for receiving a plurality of test strips, preferably the said raised ridges being discontinuous, with a first portion of the said raised ridges in contact with a first heat sink and a second portion of the said raised ridges in contact with a second heat sink.

18. An amplification processing station characterised in that it comprises a dual chamber reaction vessel as claimed in claim 10, the said arms reciprocating from a first position to a second position and the said arms, in the said second position, opening the said conduit portion.

19. A test strip characterised in that it comprises a reaction vessel as claimed in any of claims 1 to 12 and, optionally, an optical cuvette for conducting an optical analysis of a sample.

20. A test strip for a nucleic acid amplification reaction characterised in that it comprises a body portion defining a plurality of wells arranged in a row and having a first end and a second end, the said second end having a cuvette for conducting an optical anal-

ysis of a sample, and an aperture provided in the said body portion adjacent to said first end of said test strip for receiving a disposable amplification reaction vessel, preferably a vessel as claimed in any of claims 1 to 12.

21. A method for conducting a nucleic acid amplification reaction characterised in that it comprises:

providing a dual chamber reaction vessel comprising first and second chambers capable of being placed in fluid communication, the said dual chamber reaction vessel being loaded with one or more amplification reagents in the said first chamber and one or more amplification enzymes in the said second chamber;

sealing the said first and second chambers with the said amplification reagent(s) and amplification enzyme(s) from the environment with a membrane;

and, at the time of use of the said dual chamber reaction vessel,

piercing the said membrane with an implement and loading a sample into the said first chamber;

heating the said first chamber and the said sample to a first relatively high temperature, while maintaining the said second chamber at a second relatively low temperature;

cooling the said first chamber and a solution of the said sample and the said amplification reagent(s) from the said first relatively high temperature;

either passing the said solution from the said first chamber to the said second chamber, or introducing the contents of the said second chamber into the said first chamber;

and

amplifying the said solution either in the said second chamber or in the said first chamber by means of the said amplification enzyme;

and, optionally,

applying vacuum to the said second chamber, so as to draw the said solution through the said fluid channel from the said first chamber to the said second chamber, if the said amplification reaction is to occur in the said second chamber.

22. A method for conducting a nucleic acid amplification reaction characterised in that it comprises:

providing a reaction vessel comprising a first amplification chamber and a second chamber separated from the said amplification chamber by a heat and moisture barrier;

loading one or more amplification reagents into the said amplification chamber and one or more amplification enzymes into the said second

chamber:

loading a sample into the said amplification chamber:

heating the said first chamber and the said sample to a first relatively high temperature greater than or equal to 65 degrees C, while maintaining the said enzyme in the said second chamber at a second relatively low temperature:

cooling the said first chamber and a solution of the said sample and the said amplification reagent(s):

introducing the said amplification enzyme(s) into the said amplification chamber by forcing the said enzyme(s) through the said heat and moisture barrier into the said amplification chamber:

and

amplifying the said solution in the said amplification chamber.

23. A process for transferring a liquid within a reaction vessel comprising a first chamber, which communicates with the outside by means of a first conduit, and a second chamber, which communicates with the first chamber by means of a second connecting conduit, in the presence of an external gaseous environment at a pressure termed high pressure characterised in that it comprises:

(a) isolating the said first and second chambers from each other by closing the said second connecting conduit, the said first and second chambers being at the said high pressure:

(b) reducing the pressure in the said first chamber to a low pressure lower than the said high pressure, the said second chamber being maintained at the said high pressure:

(c) introducing a fluid sample into the said first chamber via the said first conduit, the said first conduit being placed in fluid communication with the said fluid sample, and thereafter closing the said first conduit:

(d) opening the said conduit to cause the pressure in the two chambers to become balanced at an intermediate pressure having a value between the said high and low pressures:

(e) opening the said first conduit to cause the said first chamber to be in communication with the outside at high pressure:

and

(f) transferring the said liquid from the first chamber to the second chamber via the second conduit with the pressure in the two chambers finally reaching the said high pressure.

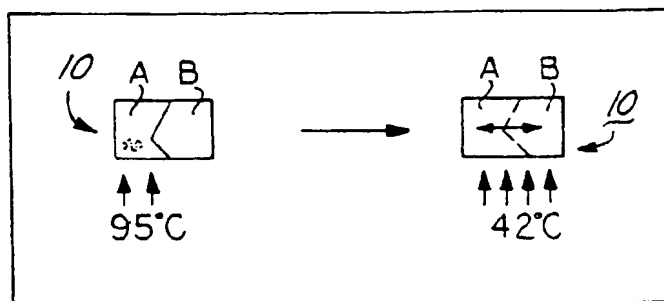


FIG. 1

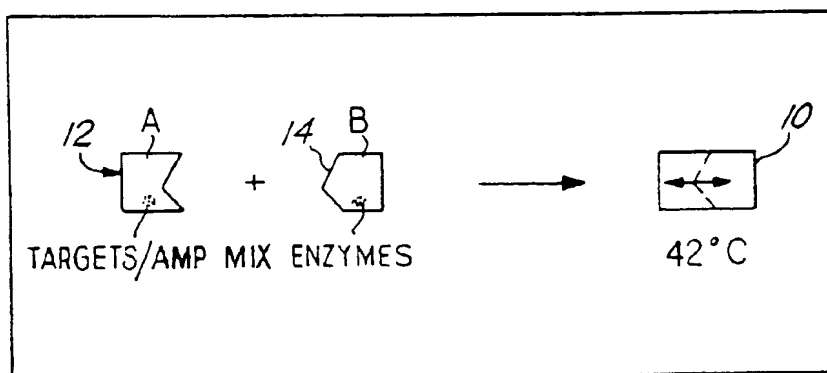


FIG. 2

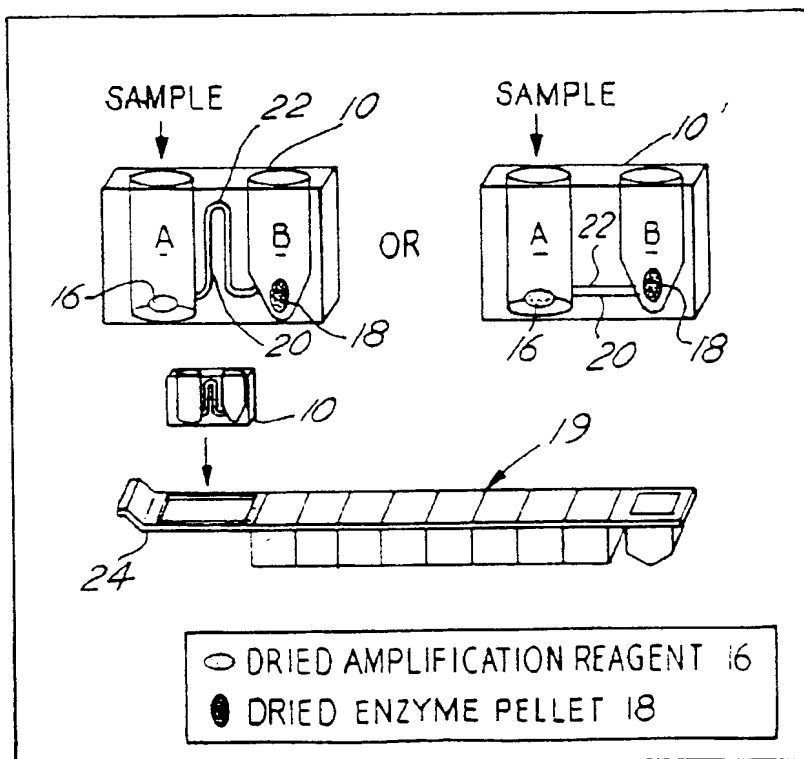
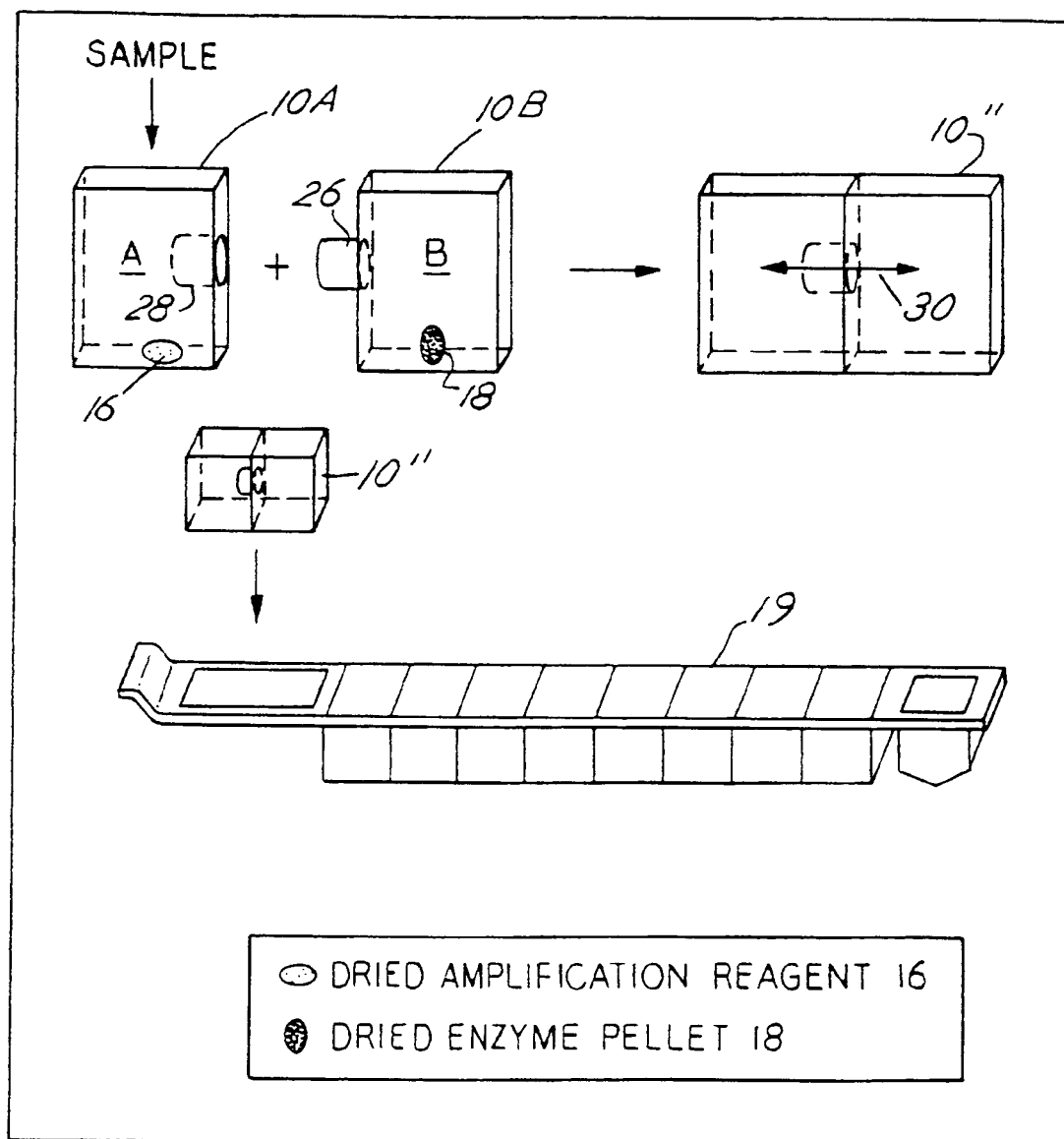
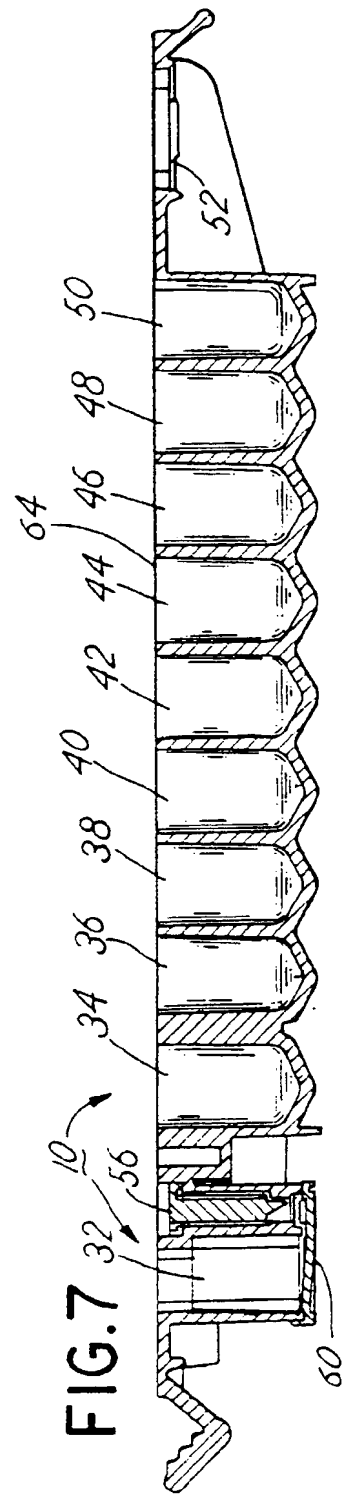
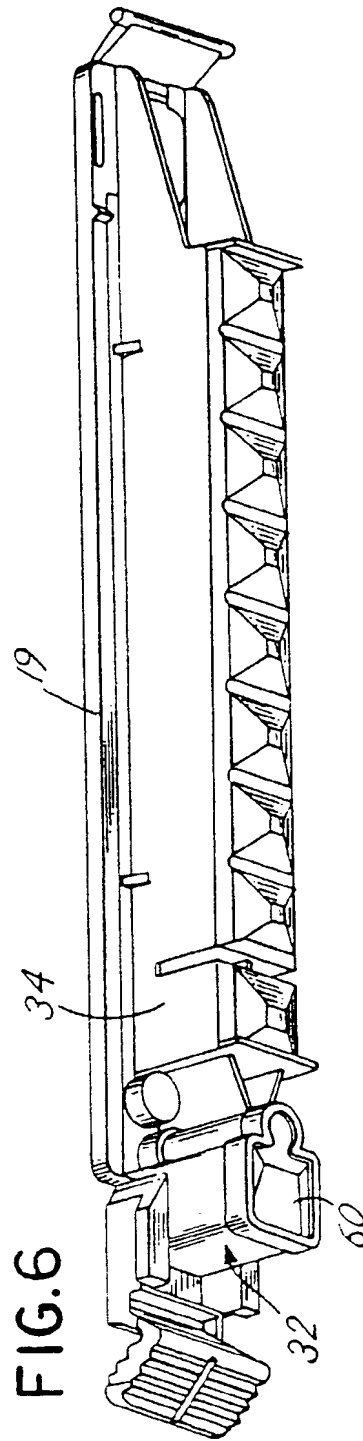
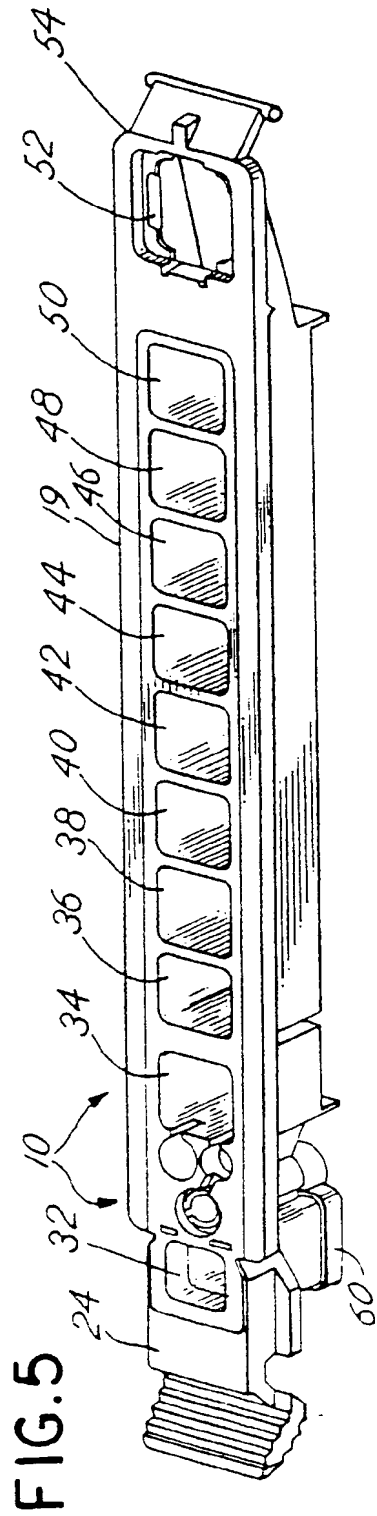


FIG. 3

FIG. 4





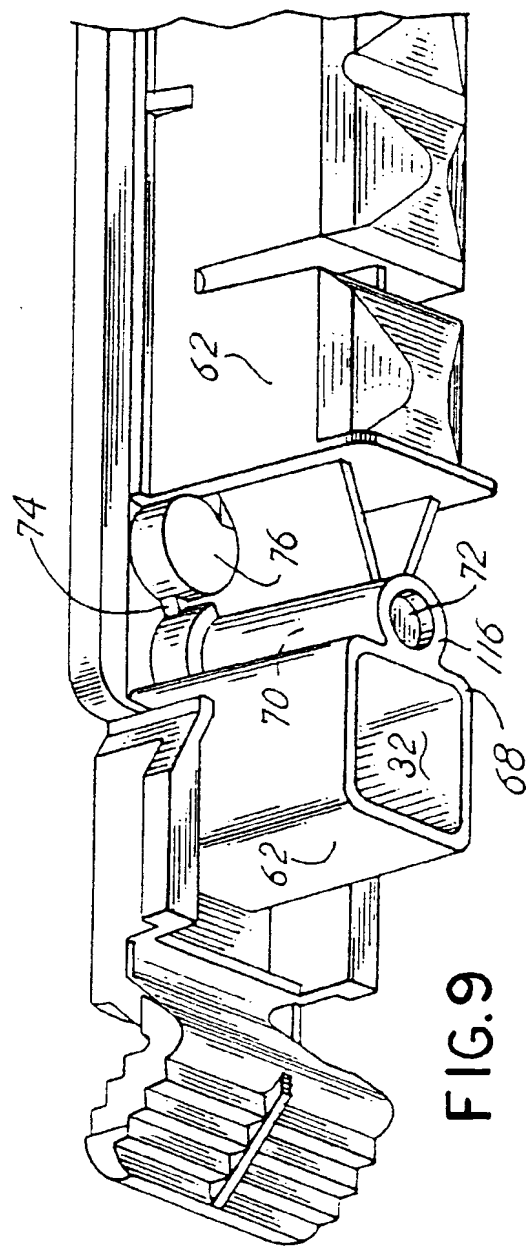
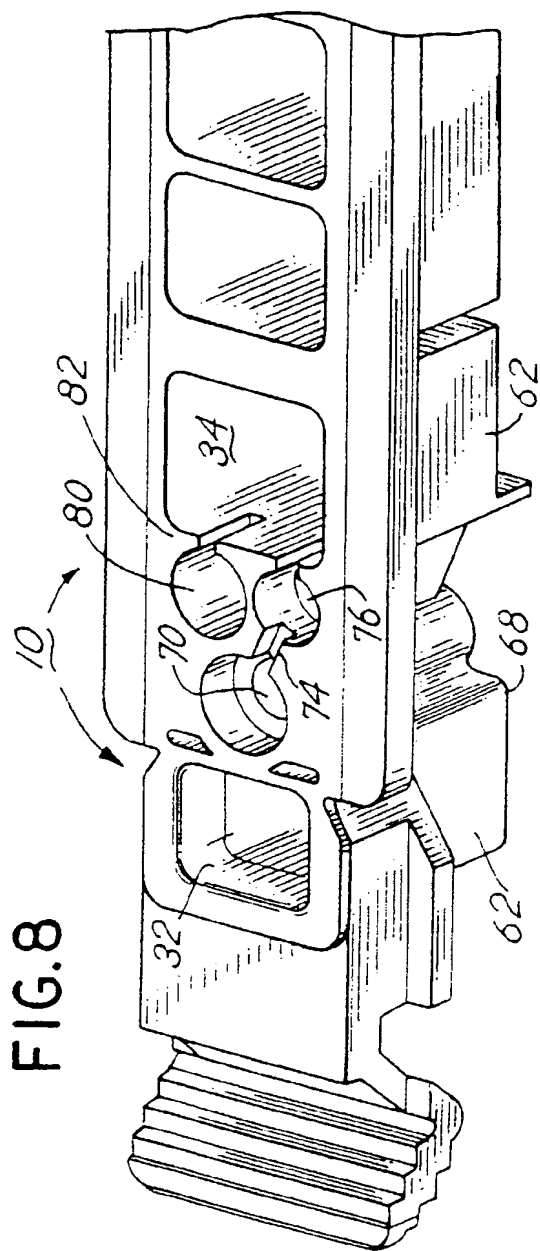


FIG.10

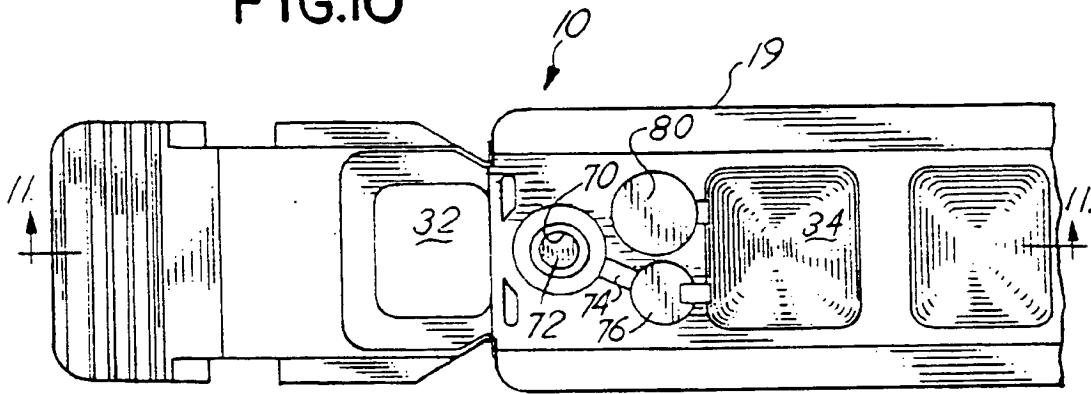


FIG.11

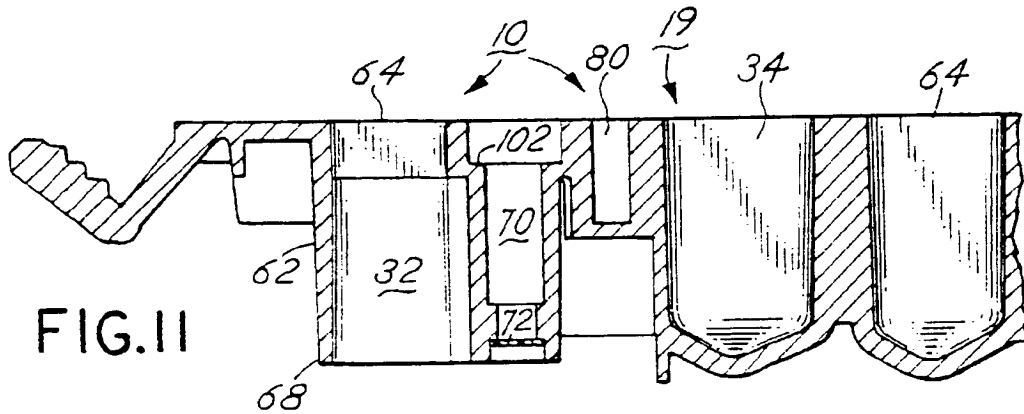


FIG.12

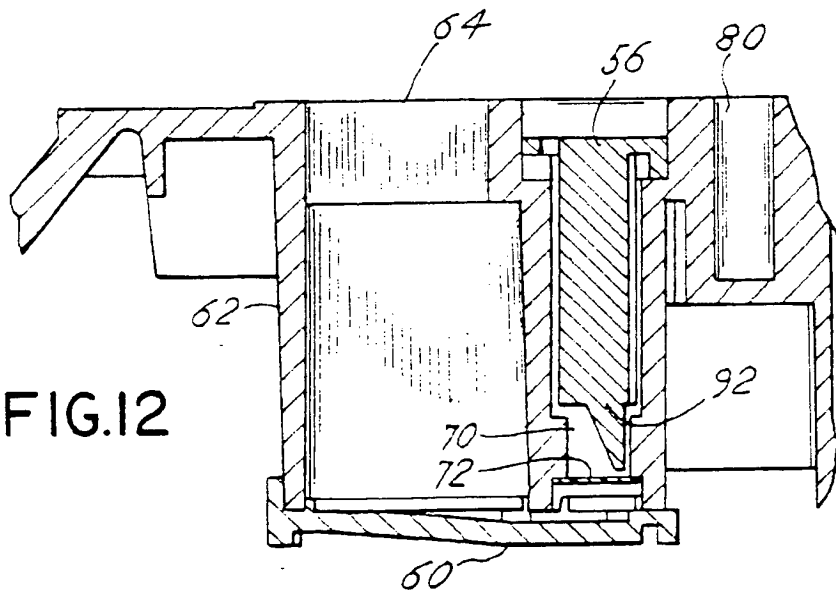


FIG.13

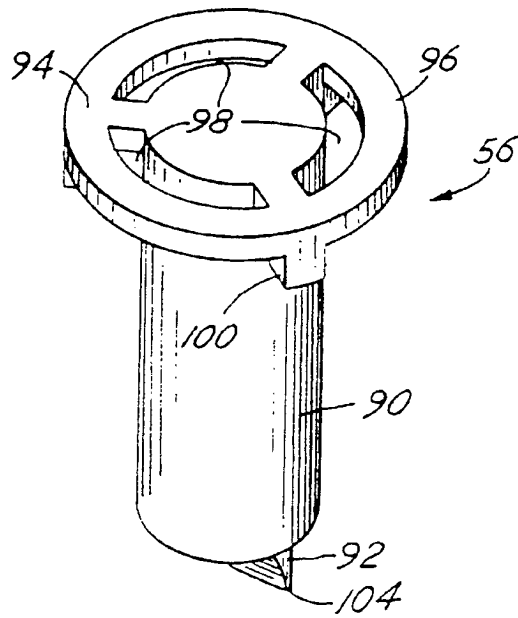


FIG.14

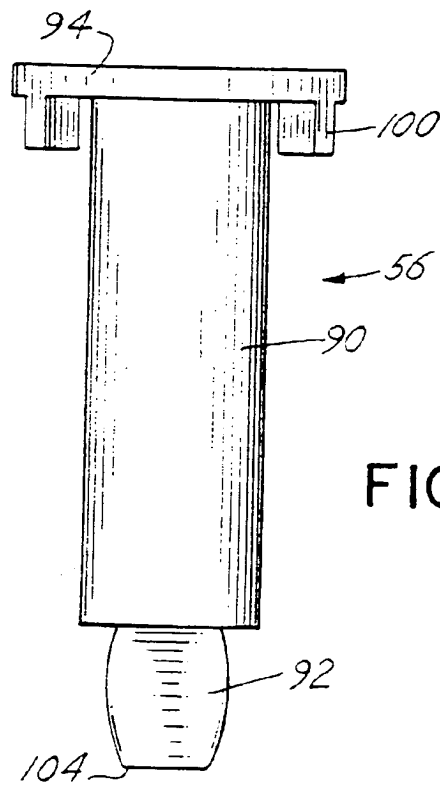
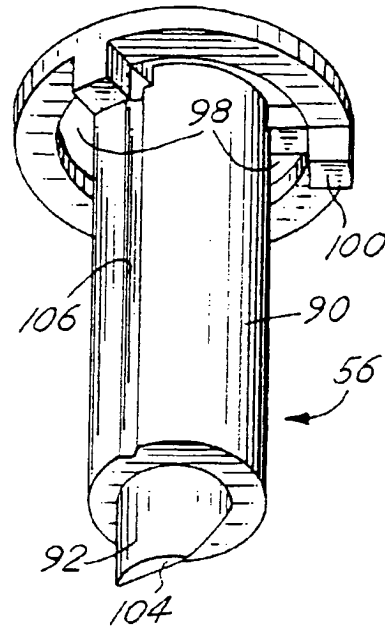


FIG.15



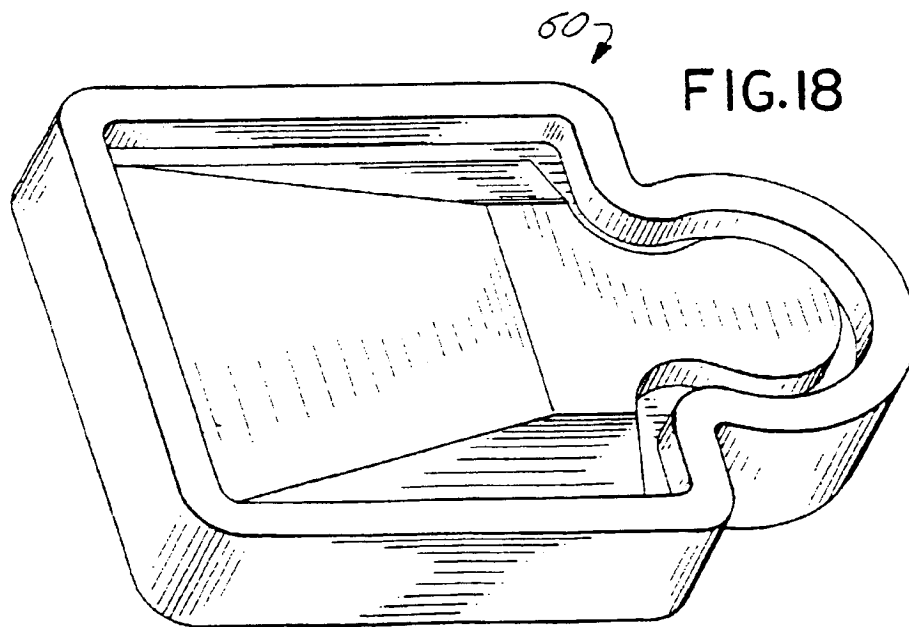
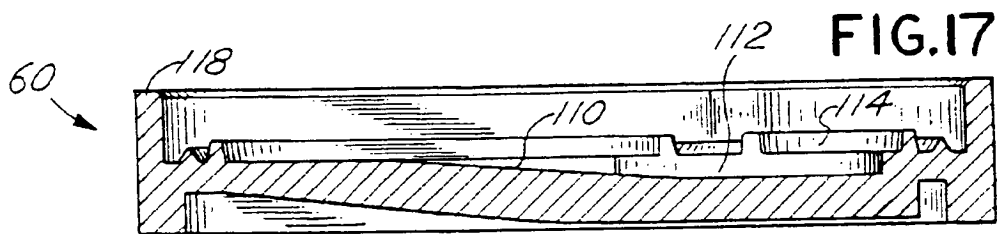
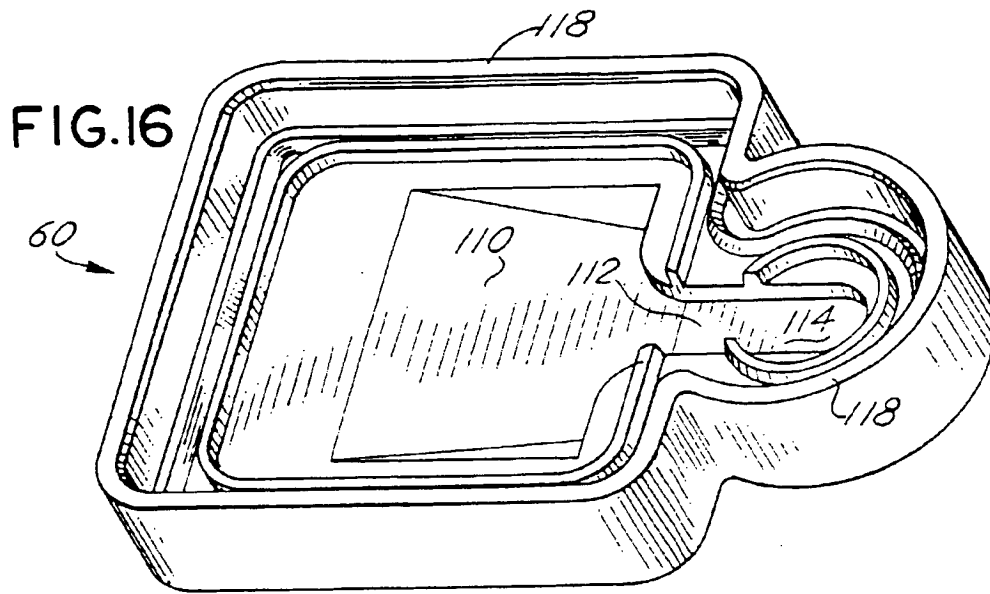


FIG.19

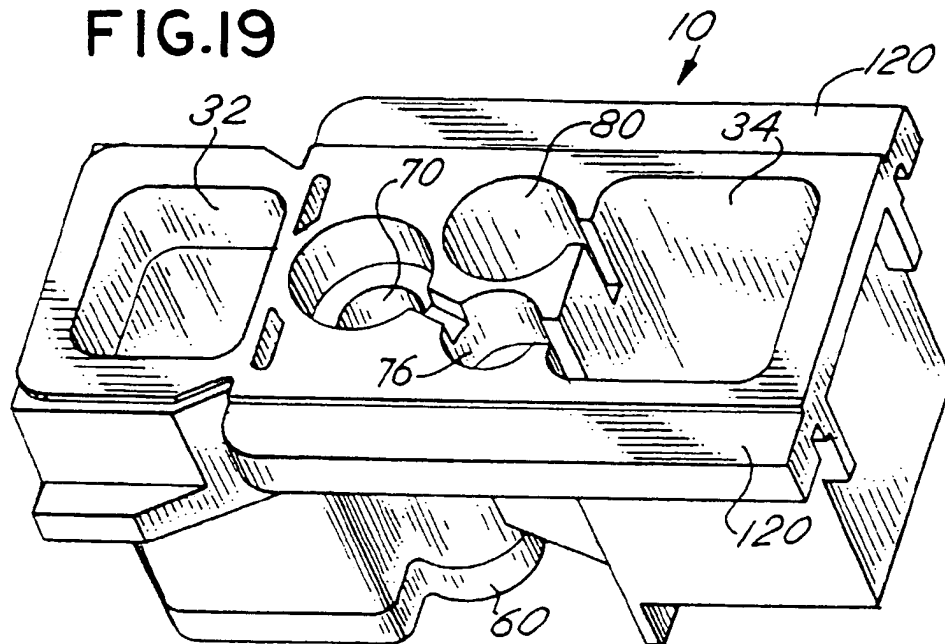
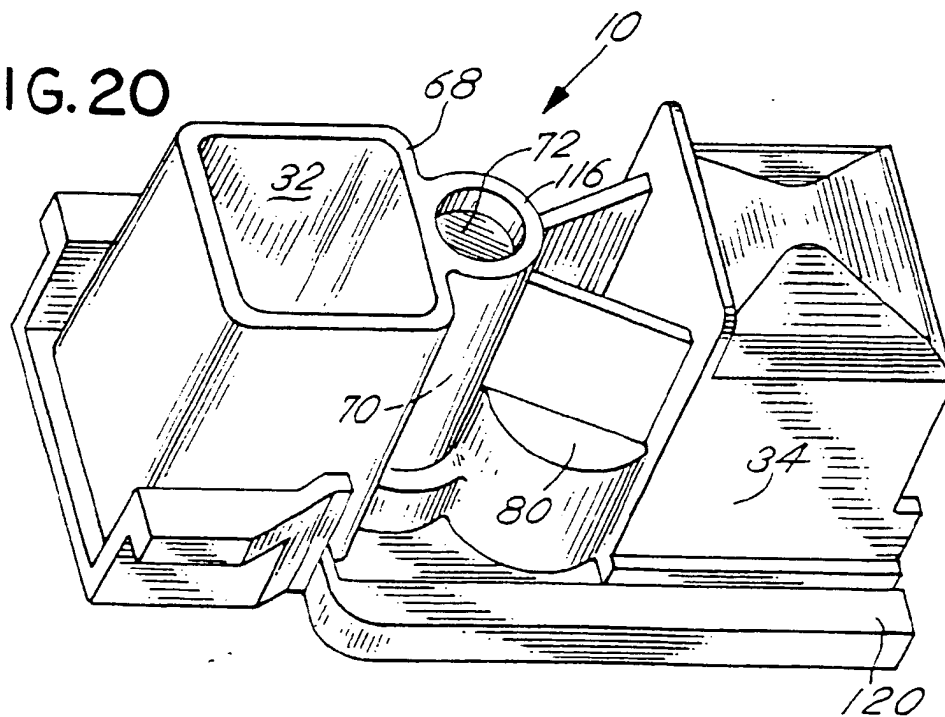


FIG.20



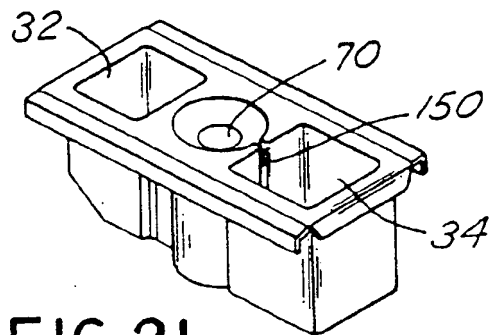


FIG. 21

FIG. 24

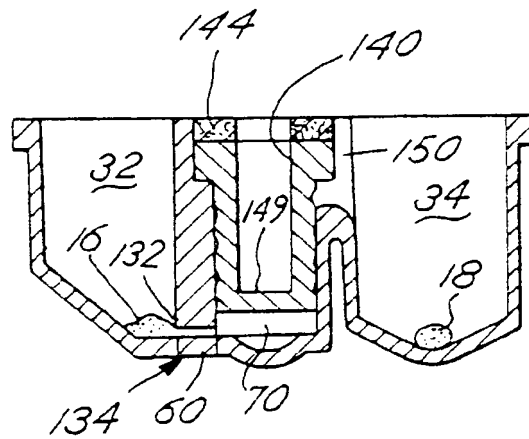
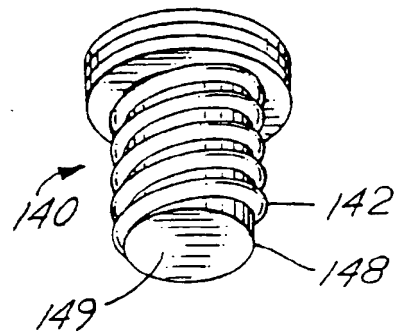


FIG. 22

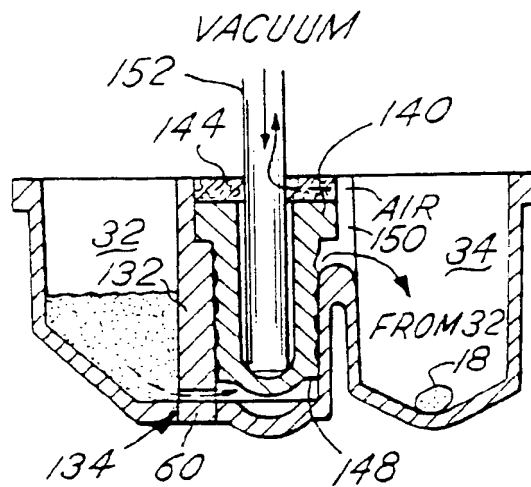
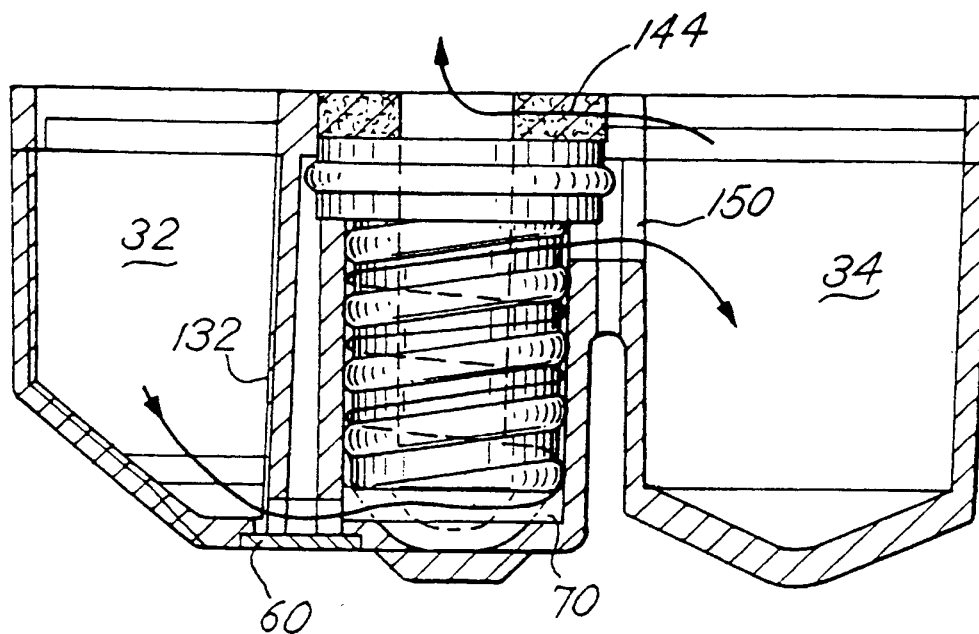
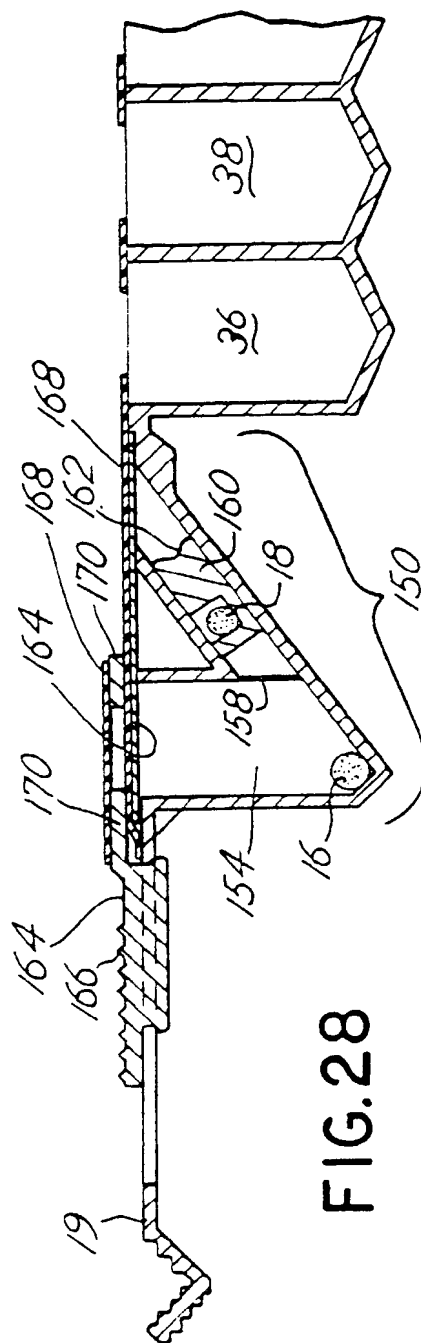
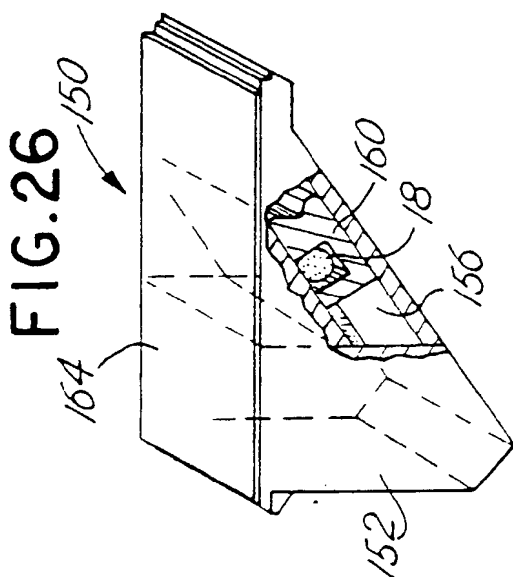
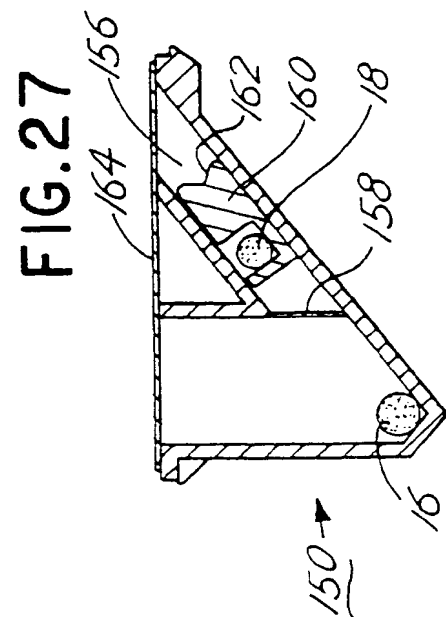


FIG. 23

FIG.25





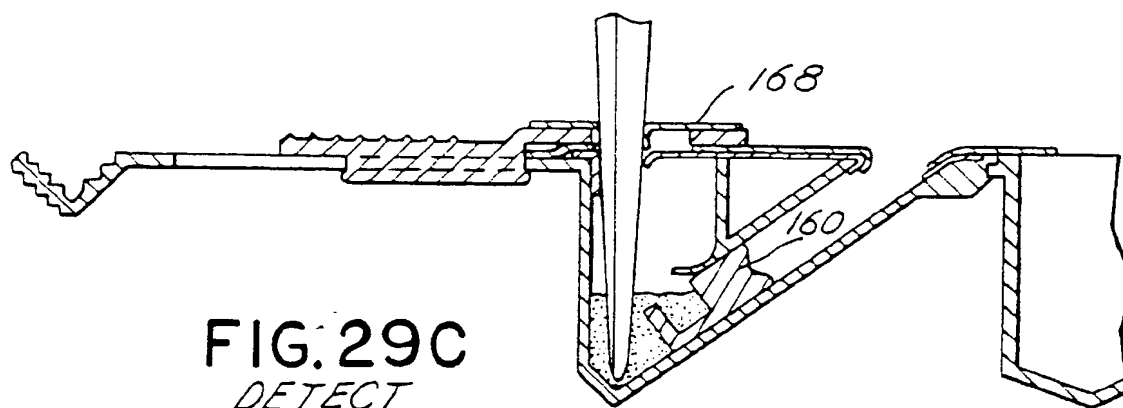
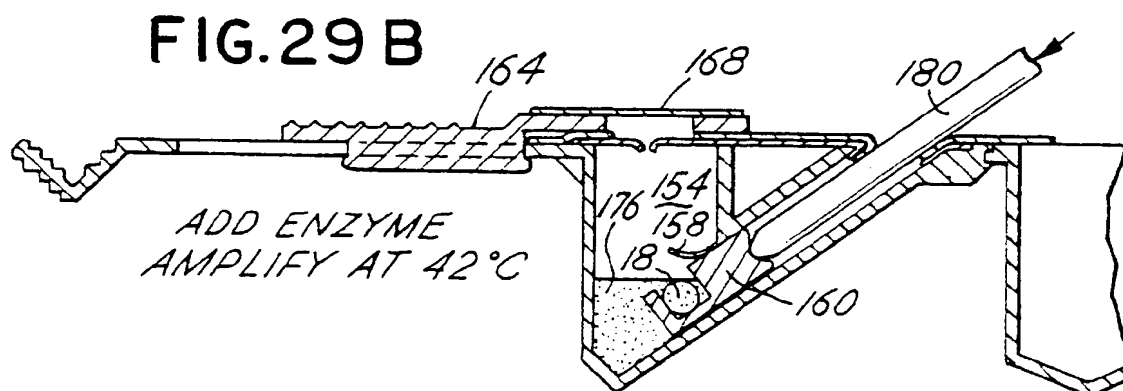
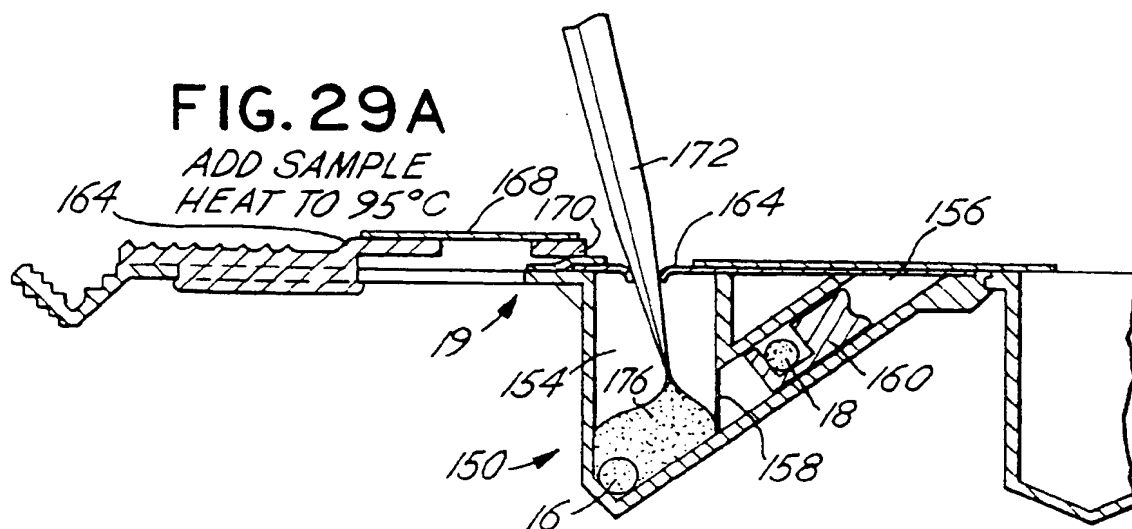
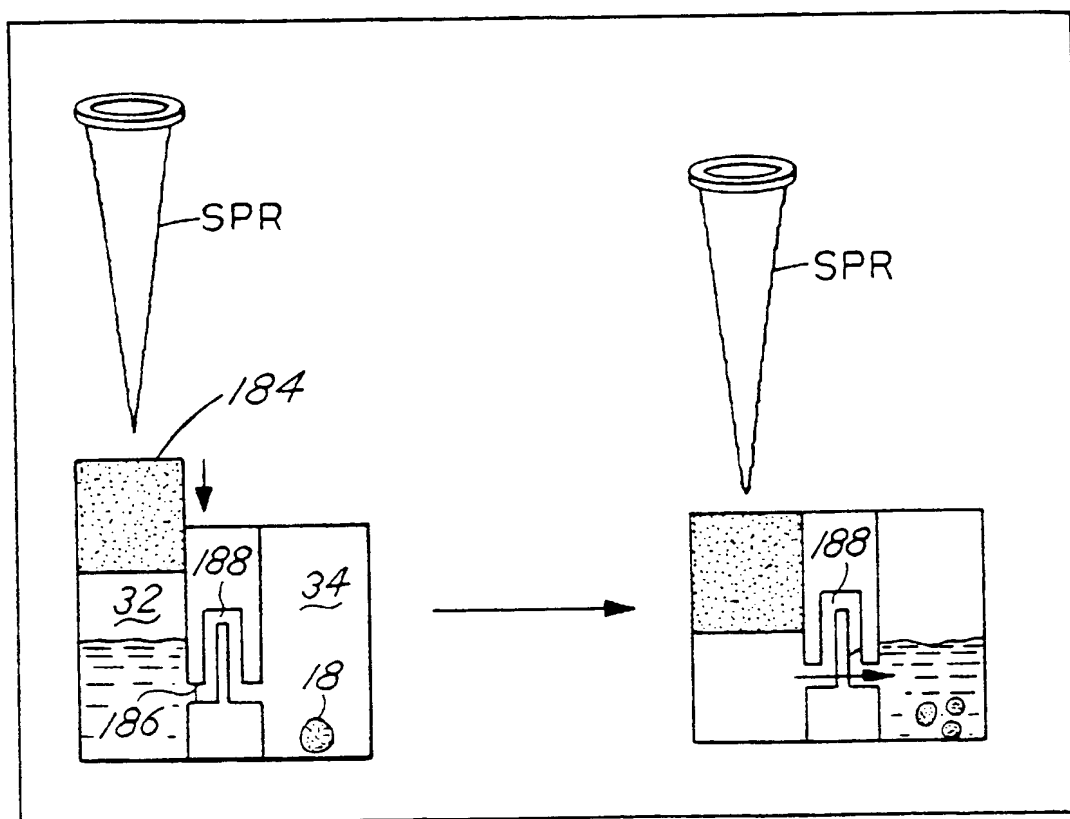


FIG.30



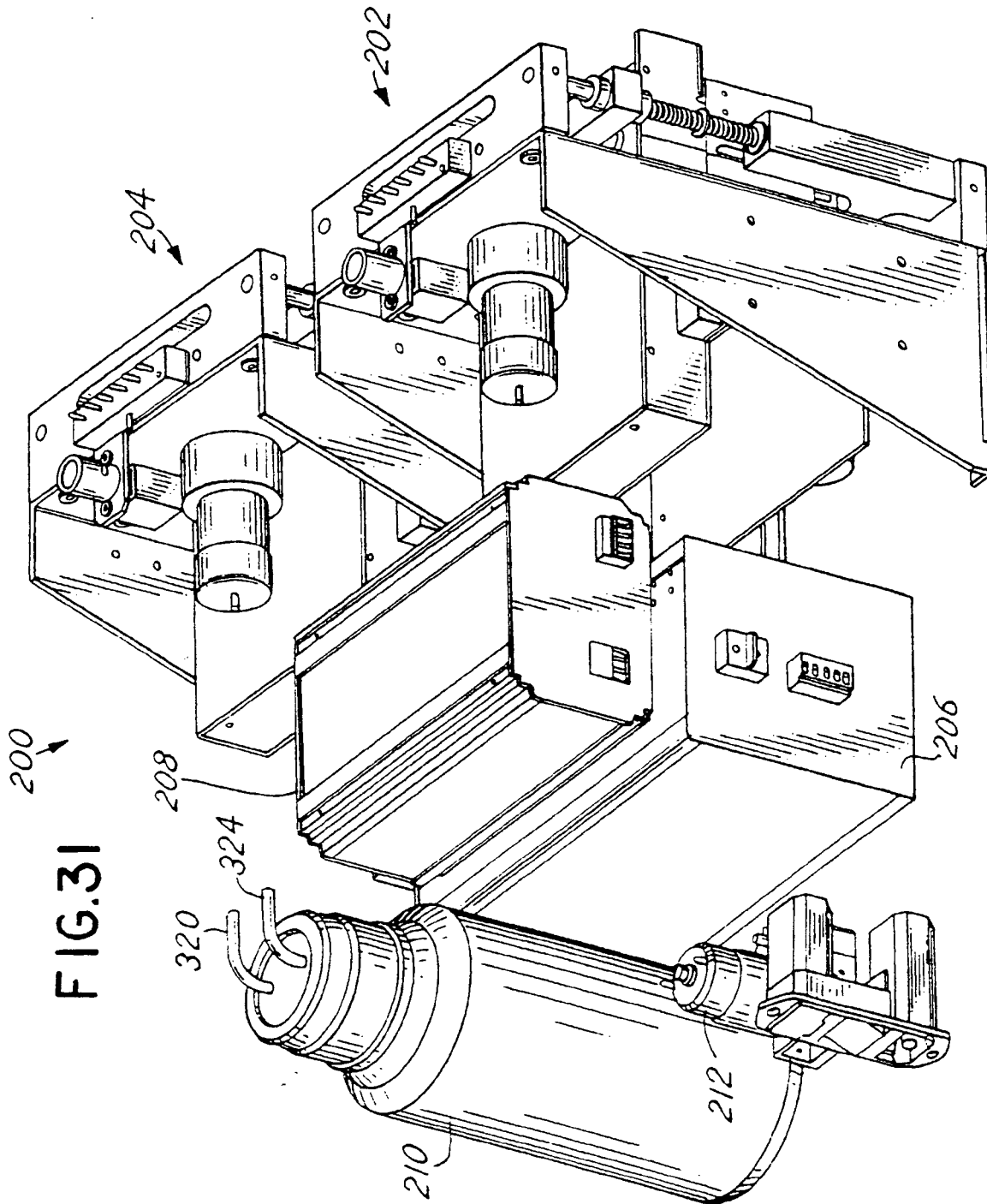


FIG.31



FIG.32

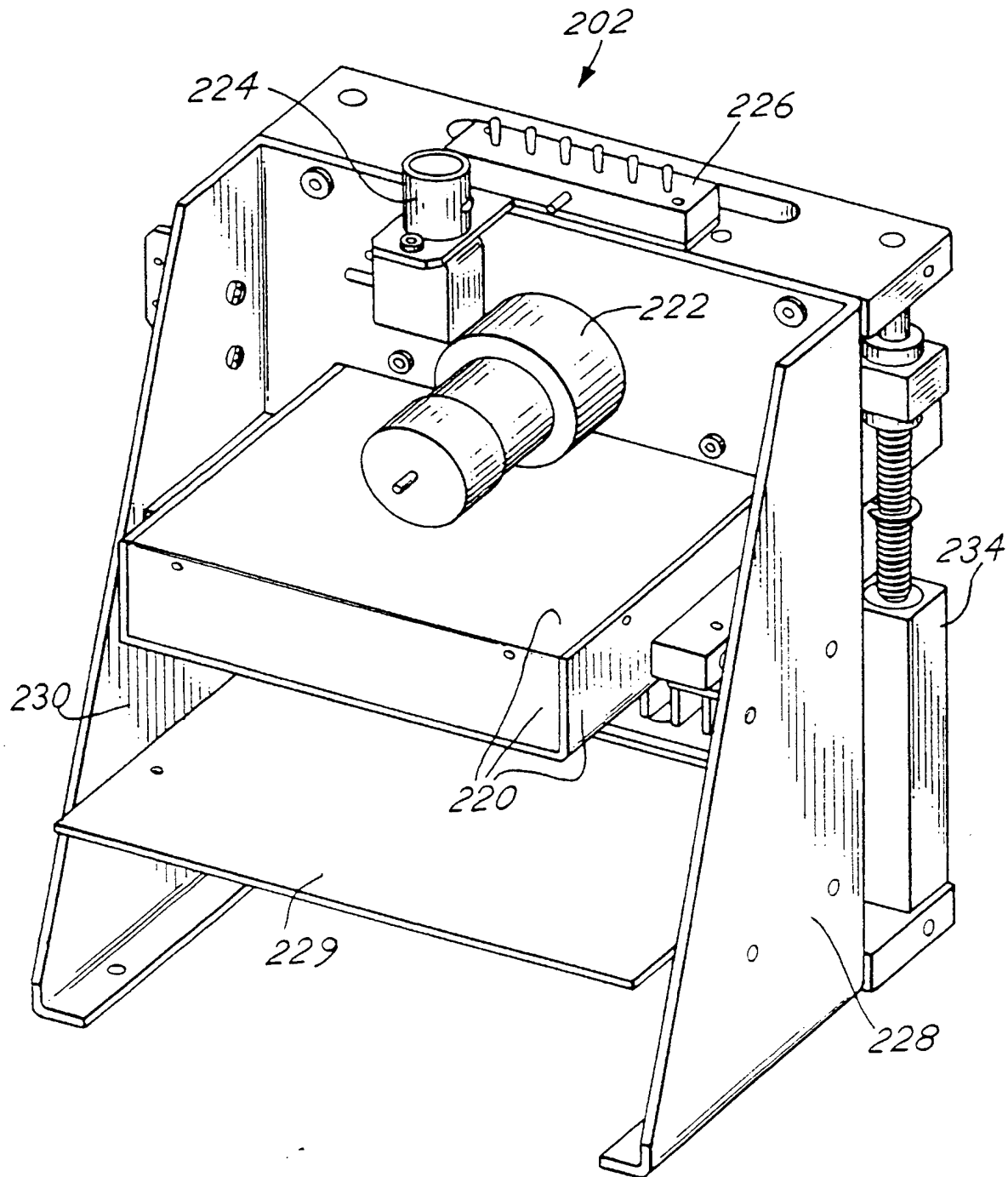


FIG. 33

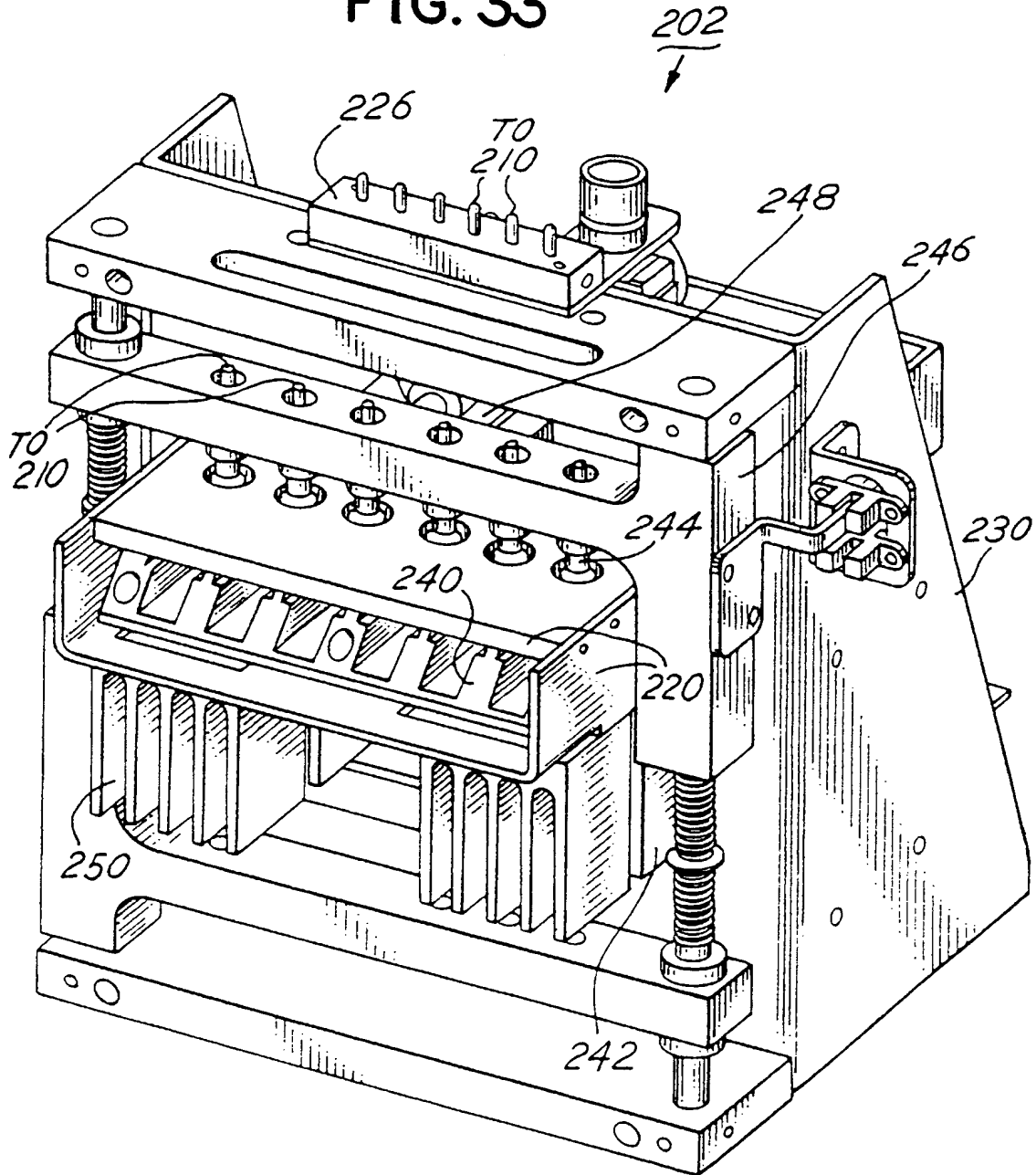


FIG.34

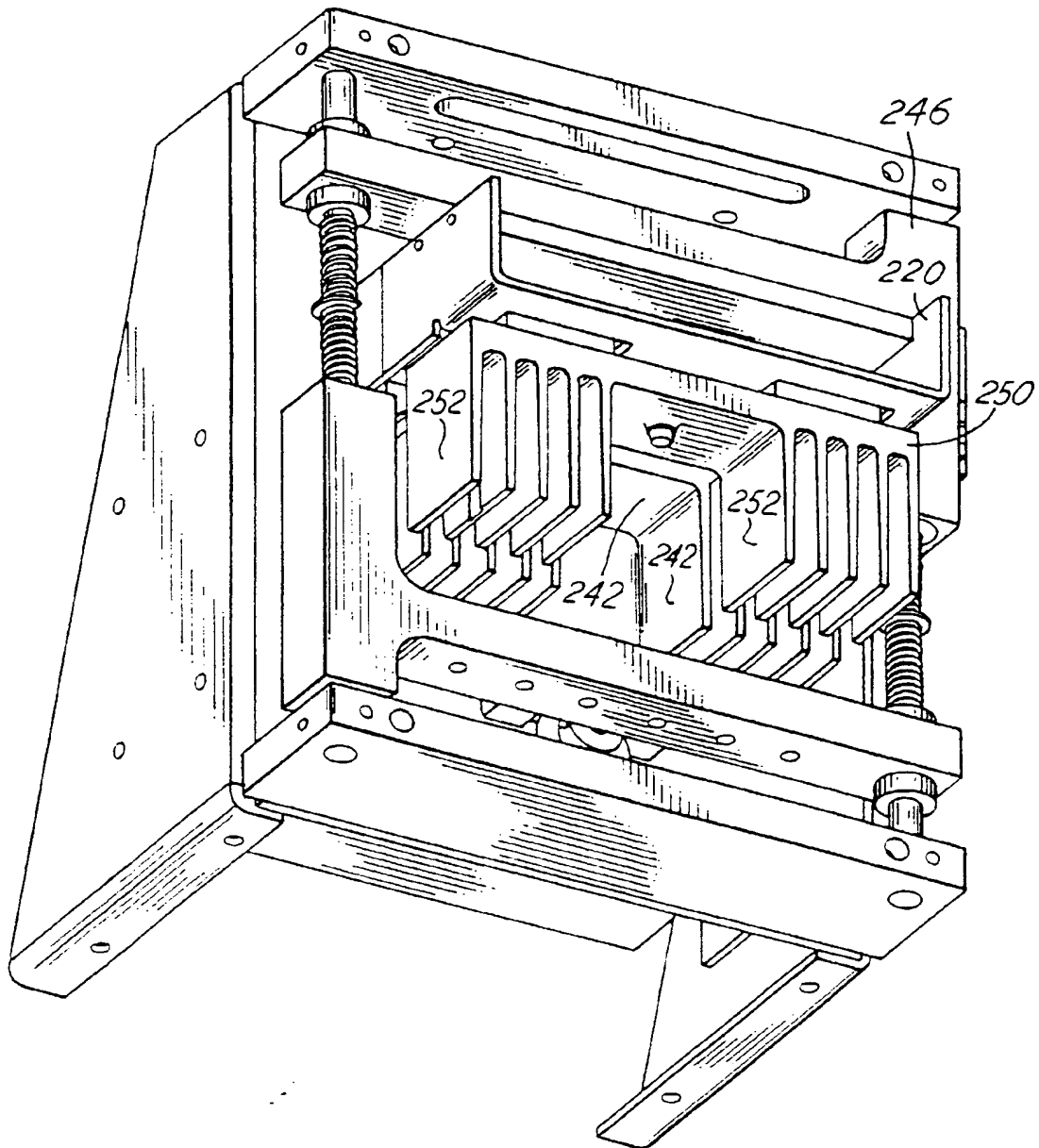


FIG.35

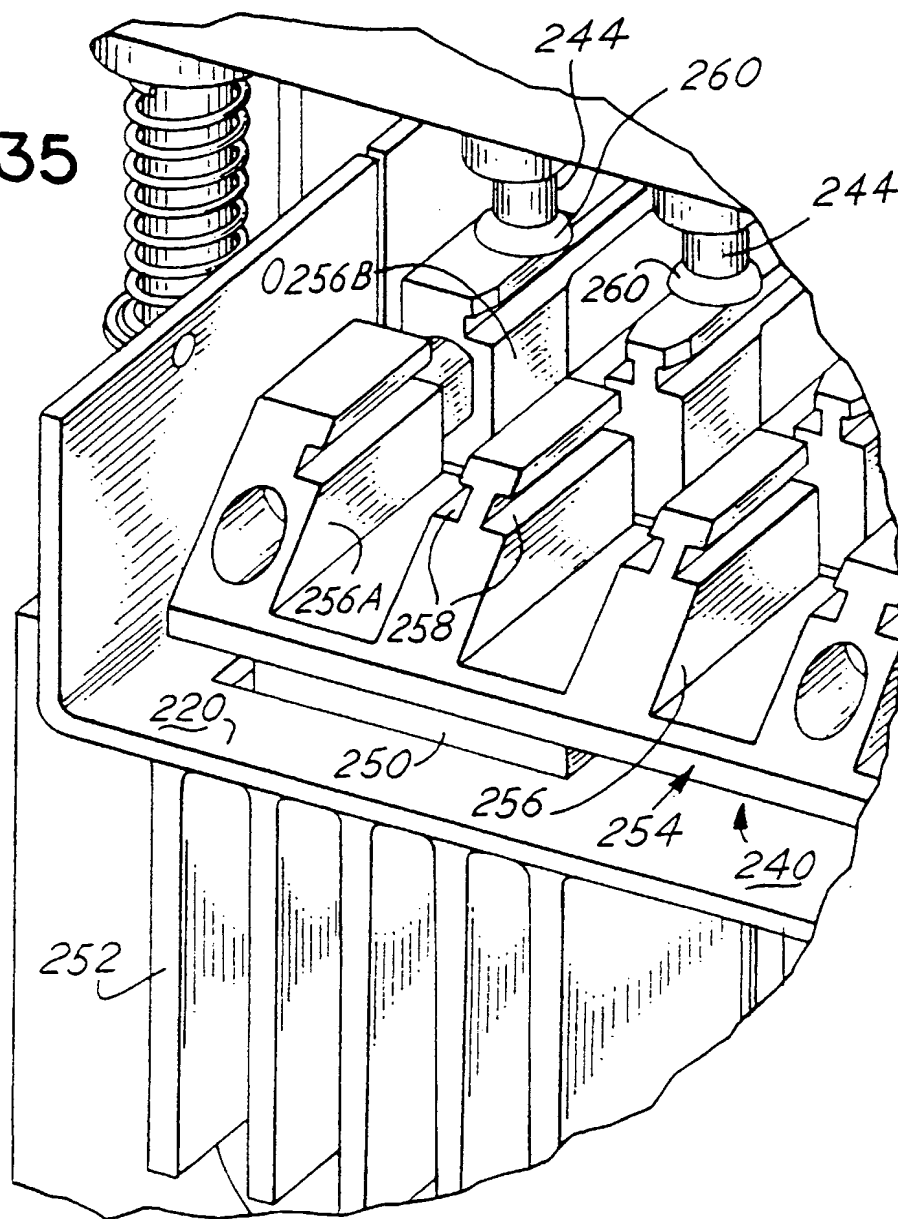


FIG.36

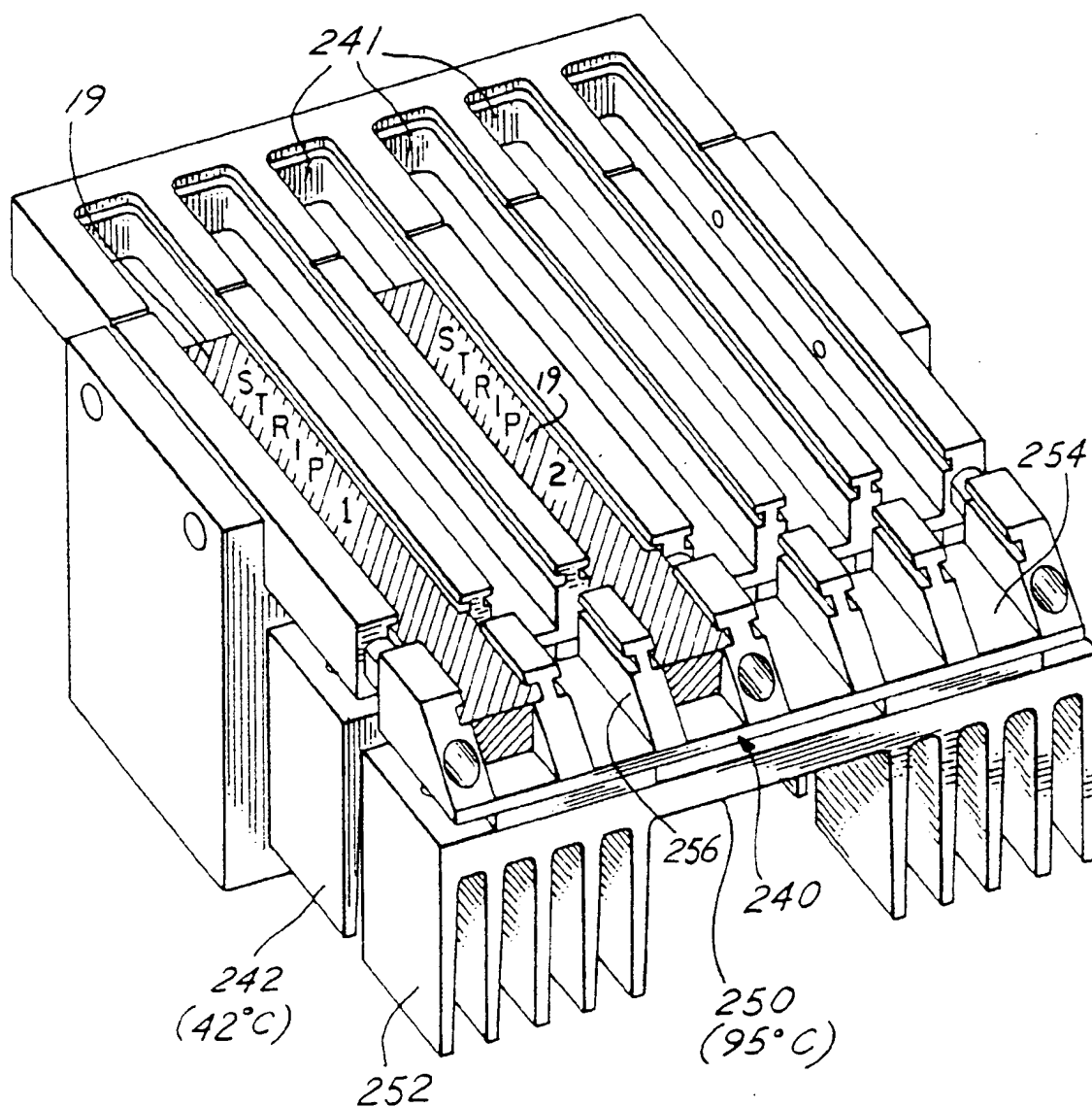
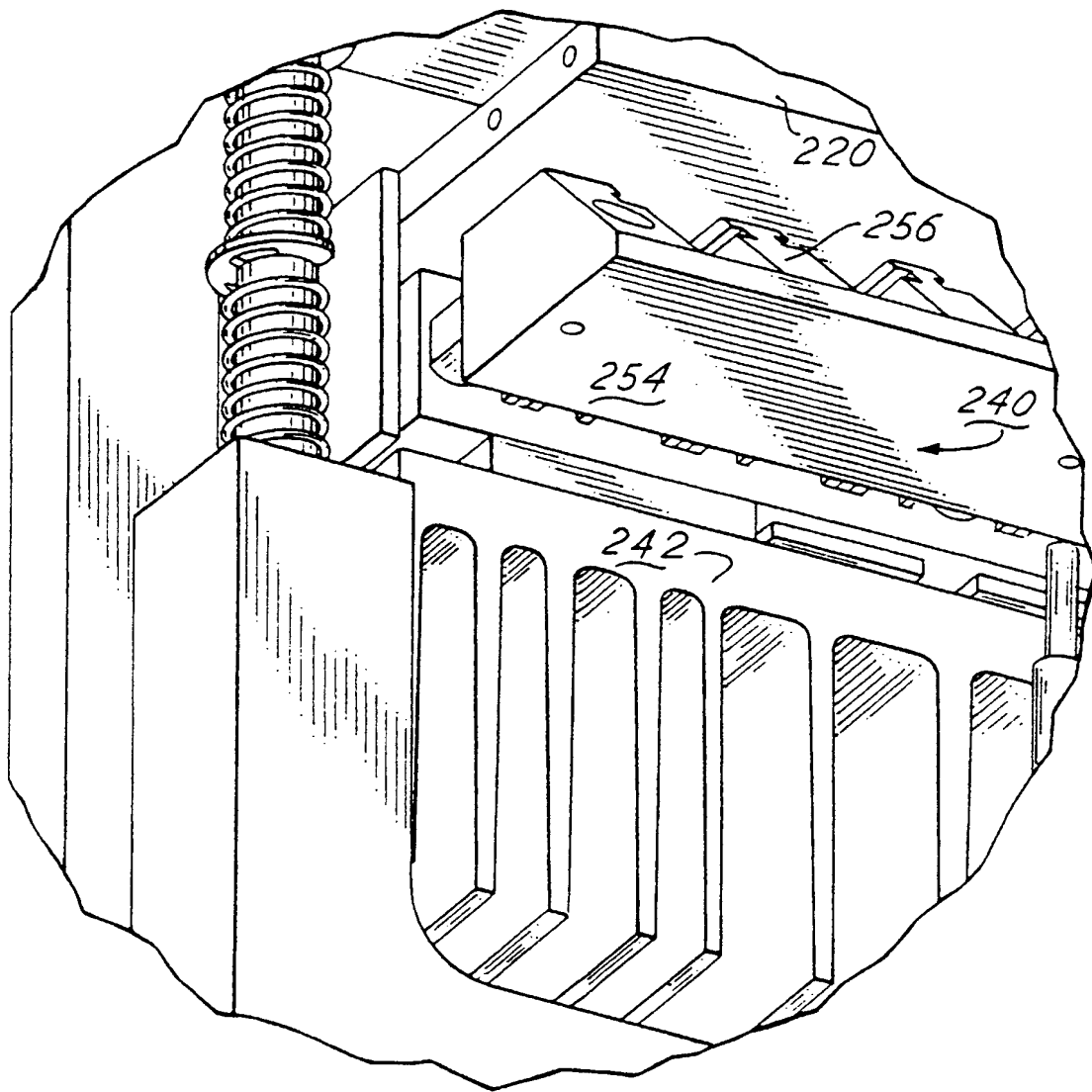
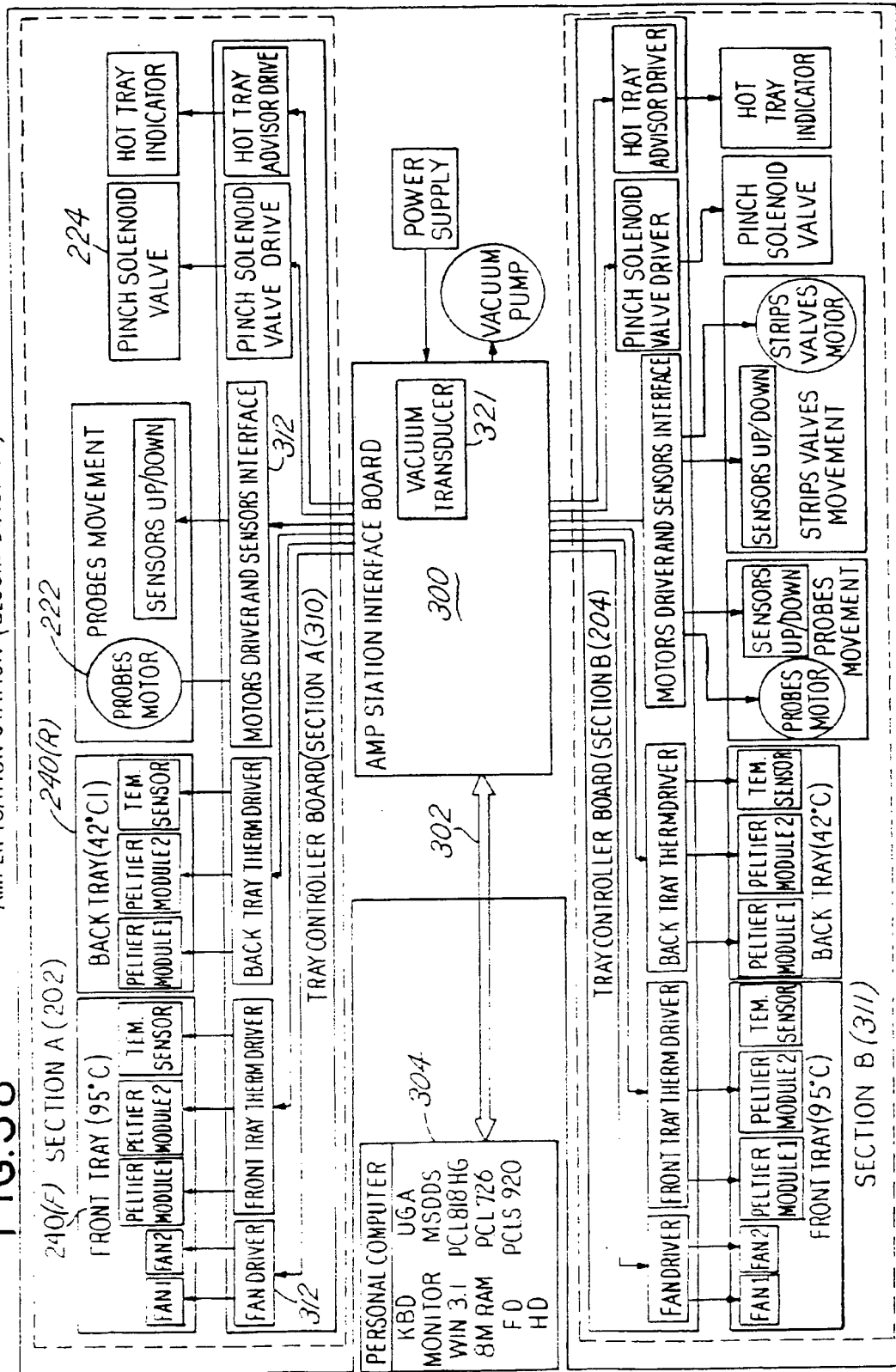


FIG. 37



# FIG.38

AMPLIFICATION STATION (BLOCKS DIAGRAM)



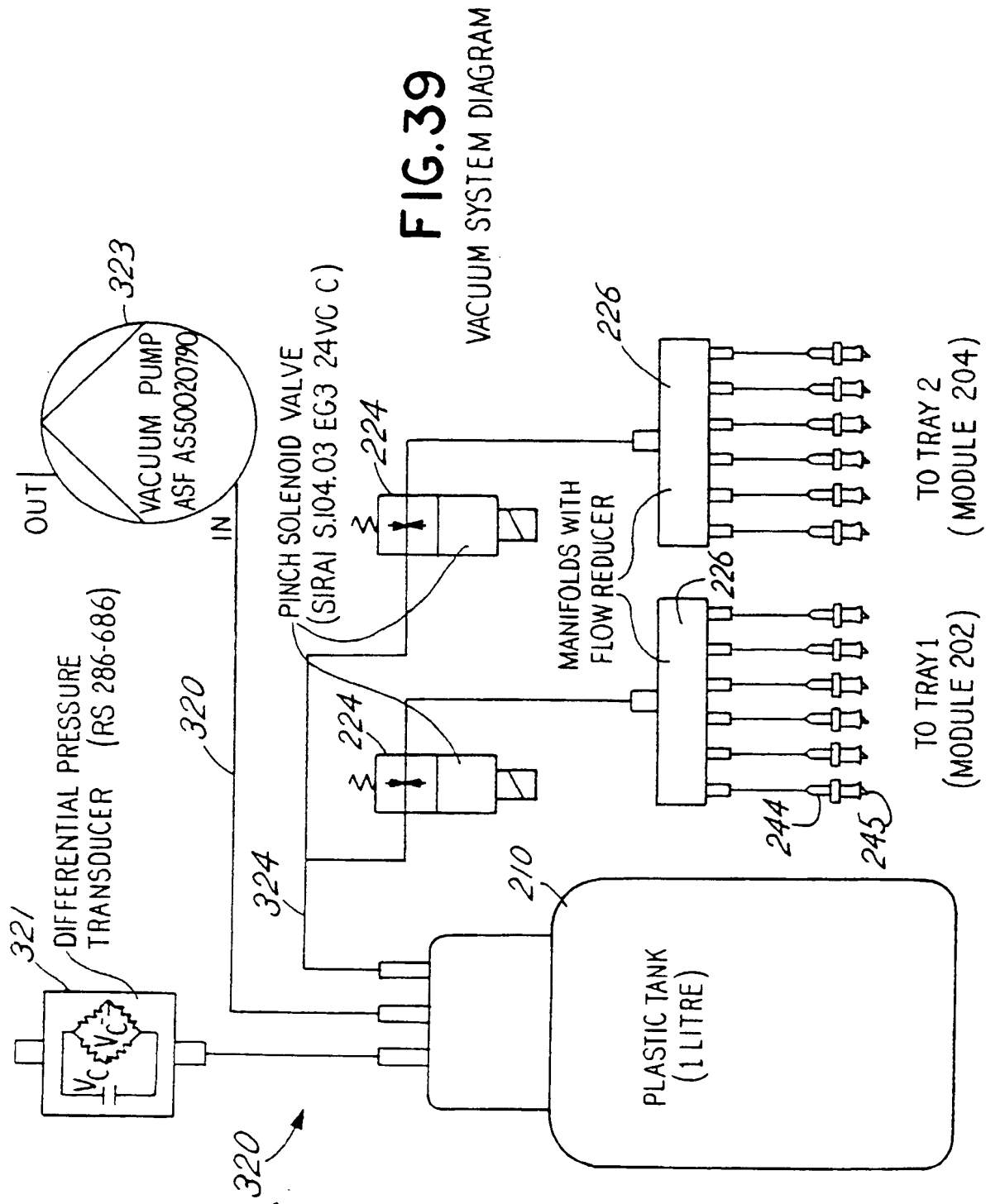




FIG.40

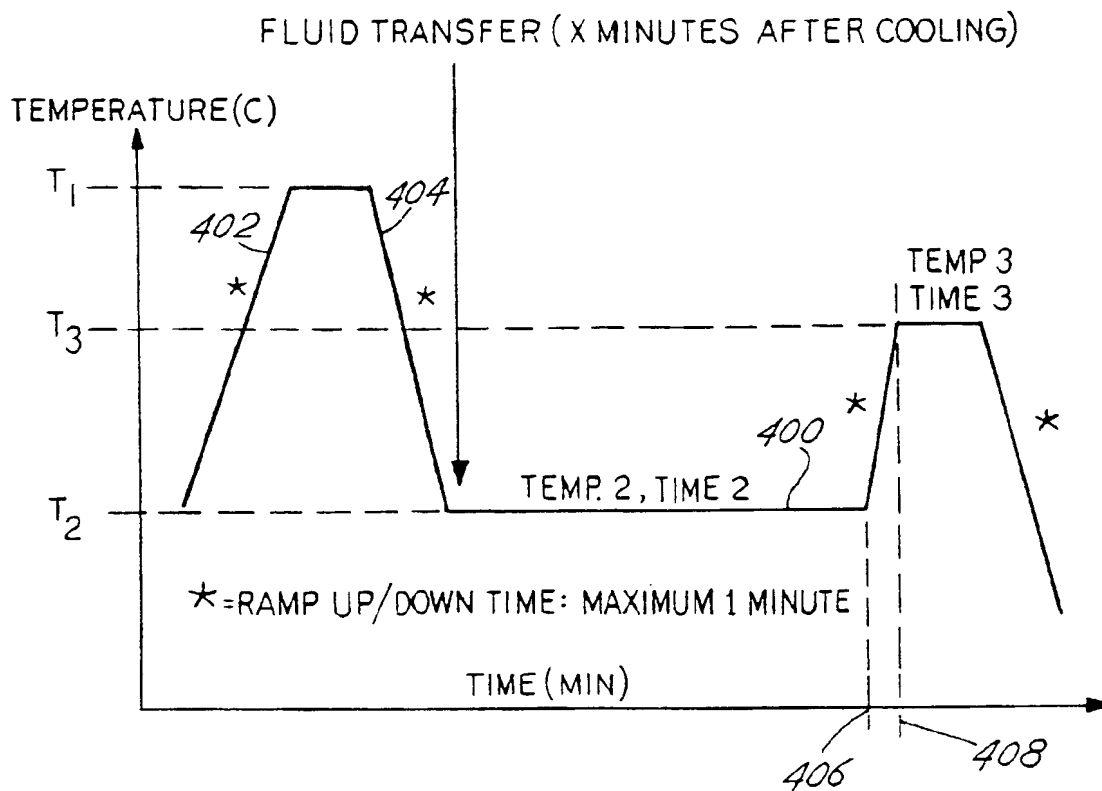


FIG.41

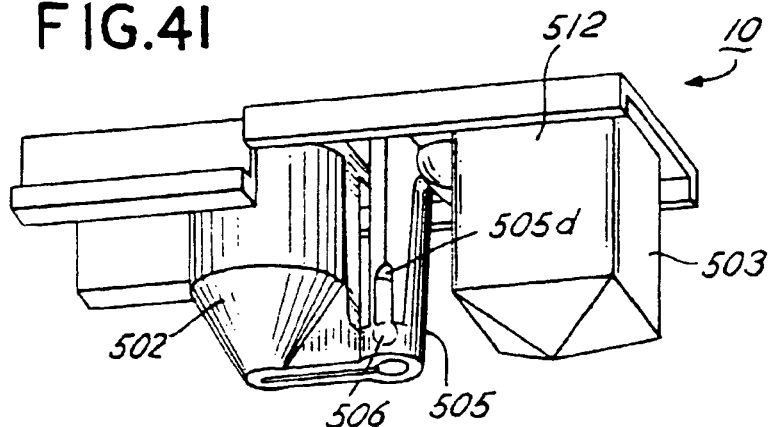


FIG.42

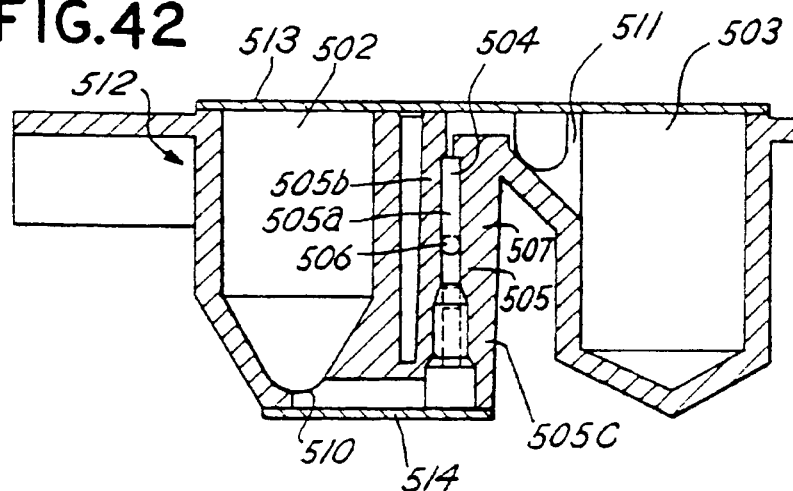


FIG.43

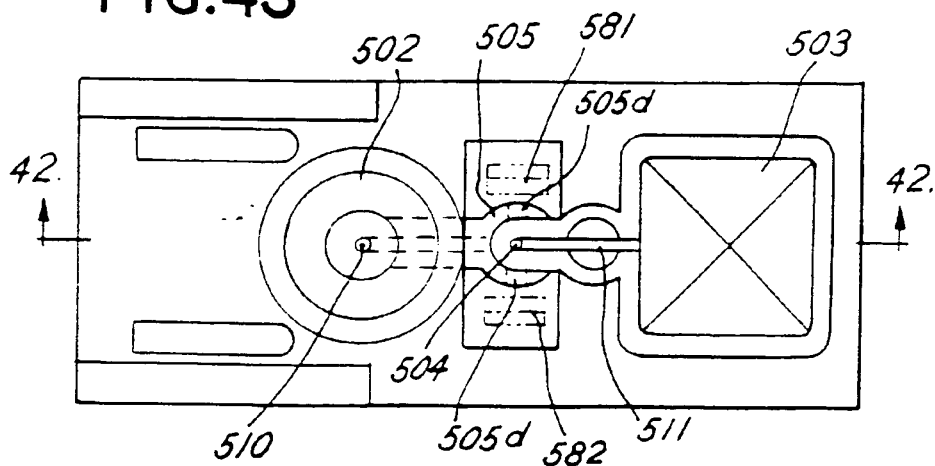


FIG.44

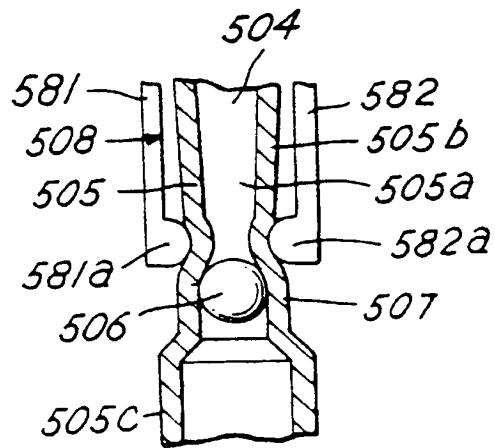


FIG.45

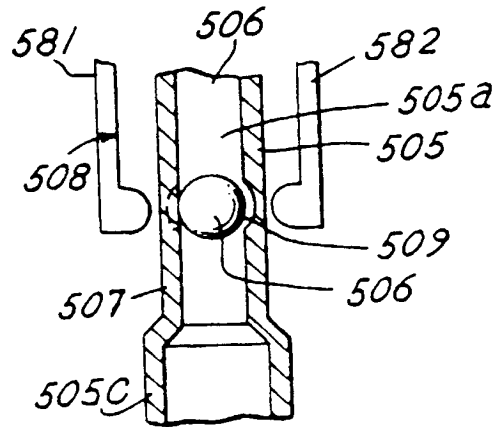


FIG.46

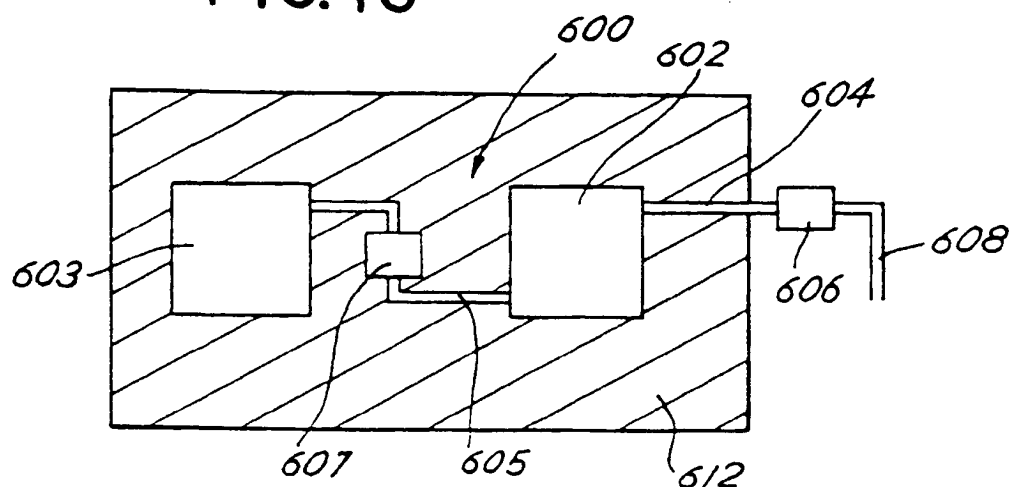


FIG.47 A

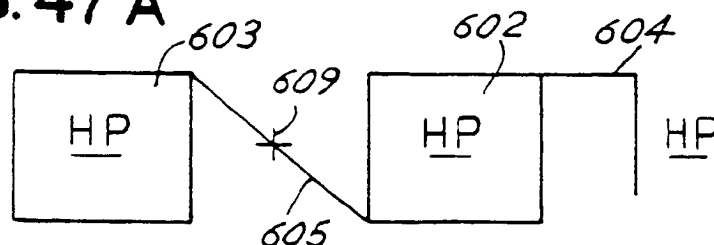


FIG.47 B

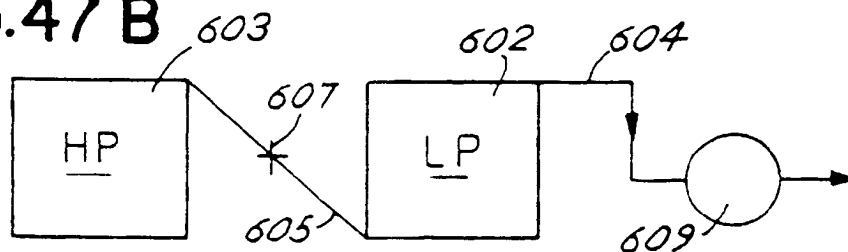


FIG.47C

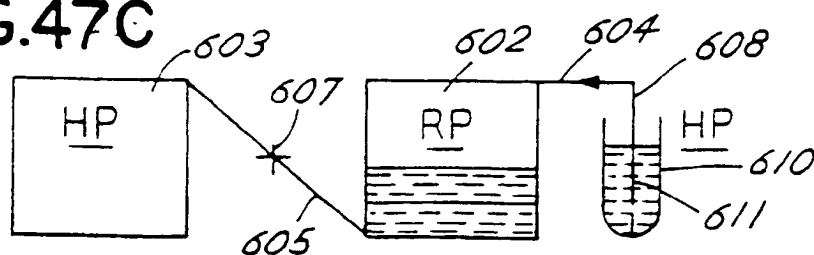


FIG.47 D

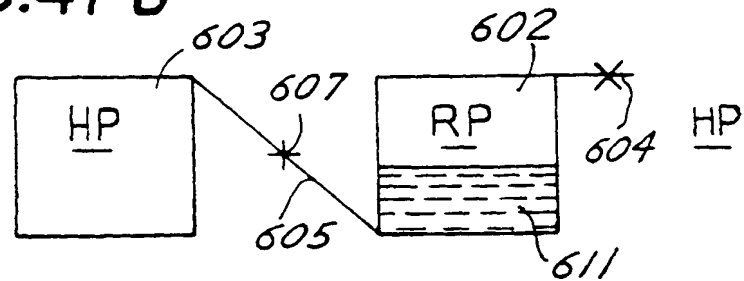


FIG.47 E

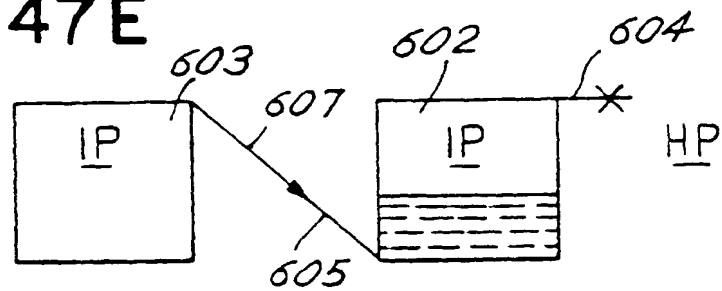
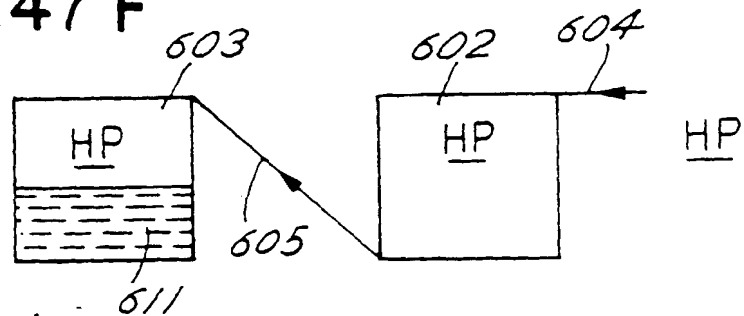


FIG.47 F





(19)



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(11)

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(12)

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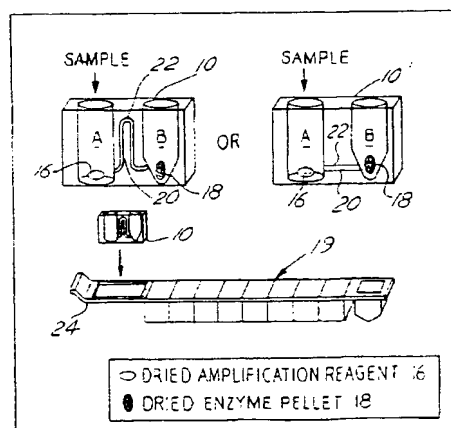
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(54) **Dual chamber disposable reaction vessel for amplification reactions, reaction processing station therefor, and methods of use**

(57) A reaction vessel for a nucleic acid amplification reaction has a first chamber containing an amplification reagent mix, a second chamber containing an amplification enzyme, and a fluid channel or chamber connecting the first and second chambers together. A fluid sample is introduced into the first chamber. After a denaturation and primer annealing process has occurred in the first chamber, the fluid chamber is opened to allow the solution of the reagent and fluid sample to flow into the second chamber. The second chamber is maintained at an optimal temperature for the amplification reaction.

A station is described for processing test strips incorporating the reaction vessels. The station includes temperature and vacuum control subsystems to maintain proper temperatures in the reaction vessel and effectuate the transfer of the fluid from one chamber to the other in an automated fashion without human interven-

tion.



EP 0 875 291 A3



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 98 30 3296

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	-/--		
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>21 March 2000</b>	Examiner <b>HOCQUET, A</b>
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

EPC FORM 503 03 82 (F04C01)





European Patent  
Office

Application Number  
EP 98 30 3296

**CLAIMS INCURRING FEES**

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

**LACK OF UNITY OF INVENTION**

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☒ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 98 30 3296

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>21 March 2000</b>	Examiner <b>HOCQUET, A</b>
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

EPC 1988 Art. 150(2) (b) (i)



European Patent  
Office

LACK OF UNITY OF INVENTION  
SHEET B

Application Number  
EP 98 30 3296

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-4,11,12,19,21,22

dual chamber reaction vessel with means for fluid communication between the chambers, amplification reagents in the first chamber and enzymes in the second chamber, strip comprising the vessel, method for conducting nucleic acid amplification using the vessel.

2. Claims: 5-10,18

dual chamber reaction vessel with a flexible conduit linking the two chambers, a rigid seal piece in the conduit, and an external constricting device creating a passage in said conduit.

3. Claims: 13-17

nucleic acid amplification station

4. Claim : 20

test strip with an aperture for receiving an amplification vessel

5. Claim : 23

process for transferring a liquid between chambers by controlling the relative pressure of the chambers.



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number  
EP 98 30 3296

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
Place of search	Date of completion of the search	Examiner	
THE HAGUE	21 March 2000	HOCQUET, A	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

EPO FORM 1501 (3/92) (P04001)

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EP 98 30 3296

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ON EUROPEAN PATENT APPLICATION NO.**

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For more details about this annex : see Official Journal of the European Patent Office, No 12/82



(12)

**EUROPEAN PATENT APPLICATION**

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(51) Int. Cl.<sup>5</sup>: **G01N 35/06, B01L 7/00**

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18.12.89 US 452666

(43) Date of publication of application:  
19.12.90 Bulletin 90/51

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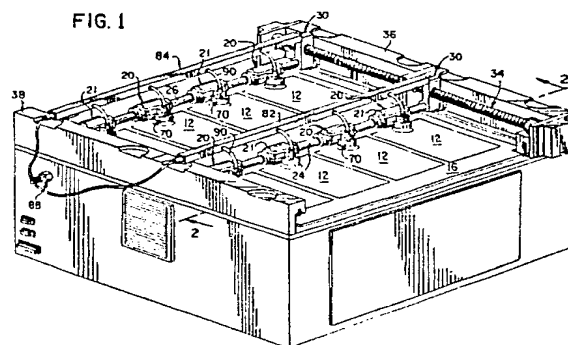
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(54) **Processing apparatus for a chemical reaction pack.**

(57) Disposable reaction packs for use in automatic analysis equipment are known. These packs comprise a body which is divided into successive chambers, each of which contains a fluid. Mixing of the fluids in such packs is achieved by manually applying pressure to each chamber of the reaction pack to open the seal between each chamber. This process is time-consuming, inaccurate and could result in damage to the reaction pack. Described herein is apparatus (10) for transferring liquid from one chamber to another in successive chambers of a chemical reaction pack (12). The apparatus (10) comprises a support surface (16) on which the packs (12) are placed, and a plurality of pressure applicators (20) which are driven to apply pressure to the packs (12). Temperature control elements (70) are also provided to heat and cool the liquid contained within each chemical reaction pack (12).

FIG. 1



**EP 0 402 994 A2**

## PROCESSING APPARATUS FOR A CHEMICAL REACTION PACK

The present invention relates to processing apparatus for chemical reaction packs, and is more particularly concerned with the progressive transfer of a test fluid between adjacent chambers of the chemical reaction pack.

Disposable reaction packs for use in automatic analysis equipment are known in the art. Such reaction packs typically comprise a body fabricated from flexible pliable material. The body of the reaction pack is divided into successive individual compartments or chambers having blister-like configurations which are normally separated from each other with seals which are rupturable or openable in response to a sufficient pressure being applied to such seals. One or more of the compartments or chambers contain predetermined amounts of reagents with which a test liquid reacts.

By applying an external linearly advancing pressure to the blister-like chambers, the normally closed seals are opened to permit transfer of the contents of a preceding chamber to a succeeding chamber. Such a technique is disclosed in US-A-3036894. The transfer of the test liquid between chambers and the intermixing thereof with the reagents is preferably accomplished without opening the reaction pack.

Manually applying an external pressure to each blister-like chamber of the reaction pack to establish sufficient internal pressure to open the normally closed seal is tedious, time-consuming, inexact, and could result in damage to the reaction pack. Inconsistency or variations in pressure and time can adversely effect chemical reactions within the pack and lead to inaccurate results. It would be beneficial, therefore, to automate the process of facilitating transfer of test liquids through a reaction pack.

The test liquids in the reaction pack may be subjected to temperature changes during the test procedure. It has been found, for example, that thermal cycling by heating and cooling a metal block on which a reaction pack is situated is relatively slow and inefficient.

Accordingly, a problem exists in providing a device which automatically transfers a test liquid through a reaction pack with consistency while facilitating heating and cooling thereof to treat the liquids contained therewithin.

It is therefore an object of the present invention to provide a device which overcomes the problems mentioned above.

In accordance with one aspect of the present invention, there is provided processing apparatus for transferring a fluid between chambers in a chemical reaction pack, the pack having blister-like

chambers formed therein, the apparatus comprising:-

a support surface for supporting at least one chemical reaction pack in a substantially horizontal plane;

at least one pressure applicator supported above the support surface for acting on an associated reaction pack to transfer fluid between adjacent chambers; and

operating means for moving each pressure applicator through a range of movement extending across the associated reaction pack;

characterized in that the operating means comprises a shaft having at least one pressure applicator arranged thereon, the shaft advancing each pressure applicator across the support surface while maintaining a substantially constant orientation between each pressure applicator and the support surface.

In accordance with another aspect of the present invention, there is provided processing apparatus for transferring a fluid between blister-like chambers in a chemical reaction pack comprising: a support surface for supporting a plurality of the reaction packs in side-by-side and end-to-end relation relative to each other;

at least one roller supported above the support surface and in alignment with the blister-like configurations on the reaction packs; and

drive mechanism means for moving the rollers across the support surface to operate against the reaction packs and facilitate transfer of fluid there-through;

characterized in that the drive mechanism means comprises at least two spaced apart generally parallel shafts each having a series of the rollers arranged endwise thereon;

and in that the drive mechanism means advances the shafts with the rollers arranged thereon across the support surface while maintaining a substantially constant orientation relative to the support surface.

In a preferred embodiment, the pressure applicators include a series of rollers which are arranged endwise on a shaft forming part of the drive mechanism. The drive mechanism advances the shaft with the rollers across the support surface while maintaining a substantially constant orientation between the shaft and the support surface.

Still further, the preferred embodiments feature a drive mechanism comprising a bracket supported at one end of the shaft and an axially elongated worm gear which extends in a direction generally orthogonal to the shaft and threadably engaging the bracket. The drive mechanism further includes

a device for rotatably driving the worm gear to move the shaft. A rack and pinion arrangement is provided at opposite ends of the shaft. As the shaft moves, it is maintained generally perpendicular to the axis of the reaction packs irrespective of where the driving force is applied by the worm gear and which of the rollers runs into resistance from the blister-like chambers on the reaction pack.

To promote temperature changes of the liquids within the disposable reaction pack, temperature-controlling elements are mounted for movement in front of and with the rollers. Each temperature-controlling element includes upper and lower units. In a start position, the upper unit of the temperature-controlling element is positioned above a similar unit disposed beneath the support surface so that the two units can work together to provide the necessary heating and cooling cycle for the liquids in the reaction pack. When the rollers are moved, the upper unit is moved with the rollers and is positioned over the next blister-like chamber in the reaction pack. After the start position, all subsequent chambers in the reaction pack are treated by the temperature-control element from the top only. As will be understood, heating may either be at a constant temperature or a heating/cooling cycle.

The apparatus of the present invention allows each of the reaction packs arranged on the support surface to be treated with constant pressures on a generally flat surface for uniform time periods. Moreover, the present invention automates and simplifies handling of chemical reaction packs. When a temperature-control element is arranged in combination therewith, temperature of the test liquid in the reaction packs can be controlled during transfer of the test liquid between chambers.

The present invention will now be described by way of example only with reference to the accompanying drawings in which:

Figure 1 is a perspective view of apparatus embodying principles of the present invention and which is capable of operating upon a plurality of chemical reaction packs;

Figure 2 is a sectioned side view taken along line 2-2 of Figure 1;

Figure 3 is an enlarged view of a portion of a drive mechanism forming part of the present invention;

Figure 4 is a top plan view of the apparatus of the present invention; and

Figure 5 is an enlarged sectional view taken along line 5-5 of Figure 4.

While the present invention is susceptible of embodiment in various forms, there is shown in the drawings and will hereinafter be described a presently preferred embodiment with the understanding that the present disclosure is to be considered as

an exemplification of the invention, and is not intended to limit the invention to the specific embodiment illustrated.

Referring now to the drawings, wherein like reference numerals refer to like parts throughout the several views, there is illustrated an apparatus 10 for operating on one or more chemical reaction packs 12. Each chemical reaction pack 12 comprises a sealed hollow body formed from fluid impermeable pliable material which is separated or divided into a series of aligned chambers; each chamber having a blister-like configuration. A test liquid is contained within one of the chambers and suitable reagents are contained within other chambers.

A more detailed description of a preferred form of reaction pack is provided in co-pending European patent application filed concurrently herewith, entitled "Temperature Control Device and Reaction Vessel".

The apparatus 10 of the present invention is preferably constructed as a self-contained unit including a base assembly 14 having a supporting surface 16 for supporting the chemical reaction packs 12 thereon in a substantially horizontal plane. As illustrated in figures 1 and 4, support surface 16 is preferably sized to accommodate a plurality of chemical reaction packs 12 in side-by-side and end-to-end relation relative to each other.

Apparatus 10 includes pressure applicators 20 arranged above the support surface 16 of base assembly 14. In a preferred form, each pressure applicator 20 comprises a roller 21 located a predetermined distance above the support surface 16 for applying external pressure to a reaction pack 12 to transfer the liquid contained in the pack from one chamber to another.

Each roller 21 is moved through a range of movement extending endwise across the reaction packs by a drive mechanism 22. As best illustrated in Figure 2, drive mechanism 22 includes an elongated shaft 24 extending above and laterally across the support surface 16. A first series of rollers 21 are arranged endwise and freely rotatable on shaft 24.

To facilitate an increase in the capacity of the apparatus 10, another elongated shaft 26 (Figure 1) extends above and laterally across the support surface 16. Shafts 24 and 26 extend substantially parallel to each other and to the support surface 16. A second series of rollers 21 are arranged endwise and freely rotatable on shaft 26.

The drive mechanism 22 moves the shafts 24 and 26 with the rollers 21 mounted thereon across support surface 16. In a preferred form, the drive mechanism 22 conjointly moves both shafts 24 and 26 and the rollers supported thereon. The apparatus can be readily modified to operate shaft 24 with

a first series of rollers 21 supported thereon independently of shaft 26 which has a second series of rollers 21 supported thereon.

The drive mechanism 22 and its relative association with shaft 24 will be described in detail with the understanding that similar construction is provided for shaft 26 and/or other shafts which support rollers 21 thereon.

As illustrated in Figure 3, the drive mechanism 22 includes a non-rotatable bracket 32 supported at one end of shaft 24. An axially elongated worm gear 34 extends in a direction generally orthogonal to the shaft 24 and threadably engages and passes through bracket 32. Opposite ends of the worm gear 34 are rotatably journaled in opposite ends of an upright support 36 connected to the base assembly 14 and extending generally parallel to the worm gear 34. Another upright support 38 (Figure 2) similar to support 36 is provided at the opposite end of shaft 24. A motor 40 (Figure 4) is fixedly supported on base assembly 14 for rotatably driving the worm gear 34 in opposite directions to effect linear movement of the shaft 24, thereby moving rollers 21 through their range of movement across the support surface 16.

The drive mechanism 22 is designed to facilitate programming of the apparatus and to allow each of the rollers 21 co-equally act on each reaction pack 12 during their range of movement. To effect such ends, and as illustrated in Figure 2, drive mechanism 22 includes rack and pinion assemblies 44 and 46 arranged at opposite ends of shaft 24 and extending substantially perpendicular or orthogonal thereto.

Alternatively, packs 12 can be operated upon randomly, if so desired.

As illustrated in Figure 3, rack and pinion assembly 44 includes a gear rack 48 and a pinion gear 50 accommodated within a cavity 51 defined in support 36. Gear rack 48 is arranged endwise on support 36 and extends parallel to the worm gear 34. Pinion gear 50 is pinned or otherwise affixed to one end of shaft 24.

Similarly, and as illustrated in Figure 2, rack and pinion assembly 46 includes a gear rack 52 and a pinion gear 54 accommodated within a cavity 55 defined in support 38. Gear rack 52 is arranged on support 38 and extends parallel to gear rack 48. Pinion gear 54 is pinned or otherwise affixed to the opposite end of shaft 24.

Drive mechanism 22 further comprises bearings 56 and 58 provided at opposite ends of shaft 24. As illustrated in Figure 2, bearings 56 and 58 are entrapped between respective upper guide faces 60 and 62 defined on respective supports 36 and 38, and respective elongated lower rails 64 and 66. Each rail 64 and 66 is fixed relative to the support surface 16. Accordingly, the horizontal axis

of shaft 24 about which rollers 21 move during their range of movement is maintained substantially parallel to and in a constant orientation with the support surface 16.

The apparatus 10 of the present invention further includes a plurality of temperature-controlling elements for providing rapid temperature changes in the fluid contained within the reaction pouch. As illustrated in Figure 1, a first series of temperature-controlling elements 70 are associated with shaft 24, while a second series of temperature-controlling elements 70 are associated with shaft 26. Each temperature-controlling element 70 is efficient, inexpensive and capable of rapidly moving the temperature of 140μl of liquid from 95°C to 55°C to 70°C and back to 95°C in a time period of from 45s to 105s (0.75min to 1.75 min) with a dwell time of at least 3s at each of these temperatures.

Turning to Figure 5, in a preferred form, each temperature-control element 70 includes an upper unit 72 and a lower unit 74. Each upper unit of the temperature-control elements 70 is supported in substantially the same manner. Accordingly, only a temperature-control element 70 as mounted to shaft 24 will be described in detail. Each upper unit 72 is carried in advance of and in alignment with a pressure applicator 20. A suitable cantilevered bracket 76 serves to mount the upper unit 72 to the shaft 24. Accordingly, when the pressure applicator 20 is moved through its range of movement the upper unit 72 of the temperature control element 70 moves therewith.

As illustrated in Figure 5, the support surface 16 defines a series of apertures 80 (only one of which is shown) which permit the lower heating unit 74 of the temperature control element to provide the necessary heating cycle to a lower surface of the chemical reaction pack 12. Additional heaters (not in the path of the pressure applicators 20) are provided as necessary for areas of the reaction pack which must be maintained at substantially constant temperature to receive the liquid warmed by the upper heater unit 72.

As illustrated in Figures 1 and 2, a pair of elongated tubular members 82 and 84 extend generally parallel to and in vertically spaced relation with shafts 24 and 26 respectively. The tubular members 82 and 84 are supported at one end by the non-rotatable brackets 32 of the drive mechanism 22. Support members 86 and 87, which are carried by the respective shafts 24 and 26, vertically support the other end of tubes 82 and 84. Accordingly, tubes 82 and 84 move with their respective shafts 24, 26 as the pressure applicators 20 move through their range of movement.

Tube 82 and 84 are connected to a source of cooling air 88 (Figure 1). Moreover, tubes 82 and 84 have a series of conduits 90 (Figures 1 and 5)

which direct the cooling air from the tubes 82 and 84 to the various temperature-control elements 70 to provide the gradients in temperatures required.

Base assembly 14 further contains a computer, temperature measurement circuits, heater and motor drive circuits, and power supplies (not shown). A computer program controls functioning of the apparatus 10 and allows for changes to the operating parameters. An internal filter/regulator and solenoid valve will provide control to the air directed to the temperature-control element 70.

Transfer of the reagents in the chambers of the reaction packs is achieved by the action of the pressure applicators 20 against the blister-like chambers in the reaction packs. In the start position, the upper and lower units 72 and 74 of each temperature-control element are positioned to provide the necessary heating and cooling cycle for the liquid in the reaction pack. As the pressure applicators 20 linearly move through their range of movement, each roller 21 applies an external pressure against the reaction pack to transfer the liquid from one chamber to an adjacent chamber. Moreover, as the pressure applicators are moved, the upper unit 72 of each temperature-control unit 70 is pushed before the pressure applicator and is positioned over a succeeding chamber in the reaction pack which requires heating. After the start position, all subsequent chambers in the reaction packs are heated from the top only.

To effect movement of the pressure applicators 20 in either direction, motor 40 is suitably operated to rotatably drive the worm gear 34. Engagement between worm gear 34 and each of the non-rotatable brackets 32 in the drive mechanism 30 causes linear displacement of the brackets and thereby the shafts 24 and 26 connected thereto.

To simplify control of the apparatus 10 and to ensure that corresponding chambers in each reaction pack are activated simultaneously for an equivalent time period, drive mechanism 22 is designed such that shafts 24 and 26 and the pressure applicators 20 carried thereby maintain a substantially constant orientation relative to the support surface 16. The rack and pinion assemblies 44 and 46 associated with each shaft 24 and 26 of the drive mechanism are provided to effect such ends. Each gear rack 48 and 52 is held stationary above the support surface 26. Each pinion gear 50 and 54 which intermeshes with the racks 48 and 52, respectively, is therefore caused to rotate upon linear movement of the shafts 24 and 26 with the rollers 21 attached thereto. Because each pinion gear is affixed to an end of a respective shaft, rotational movement imparted to the pinion gear is likewise imparted to the shaft. Accordingly, each shaft is positively driven at both ends and is maintained substantially perpendicular to the longitudinal axis

of the packets regardless of where a driving force is applied thereto and regardless of which of the rollers 21 carried thereon runs into resistance from the reaction pouch.

Moreover, the bearings 56 and 58 arranged at opposite ends of each shaft 24 and 26 further promote and maintain a substantially constant orientation between the pressure applicators 20 and the support surface 16. The ability to maintain a constant orientation between the pressure applicators 20 and the support surface 16 simplifies the control of the apparatus 10 and allows each of the packs to be co-equally acted upon for equal time periods, thereby facilitating handling of the reaction packs.

### Claims

1. Processing apparatus for transferring a fluid between chambers in a chemical reaction pack, the pack having blister-like chambers formed therein, the apparatus comprising:-

a support surface (16) for supporting at least one chemical reaction pack (12) in a substantially horizontal plane;

at least one pressure applicator (20, 21) supported above the support surface (16) for acting on an associated reaction pack (12) to transfer fluid between adjacent chambers; and

operating means (22, 24, 26, 32, 34, 36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 64, 66) for moving each pressure applicator (20, 21) through a range of movement extending across the associated reaction pack (12);

characterized in that the operating means (22, 24, 32, 34, 36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 64, 66; 26) comprises a shaft (24; 26) having at least one pressure applicator (20, 21) arranged thereon, the shaft (24; 26) advancing each pressure applicator (20, 21) across the support surface (16) while maintaining a substantially constant orientation between each pressure applicator (20, 21) and the support surface (16).

2. Processing apparatus according to claim 1, wherein the operating means further comprises a non-rotatable bracket (32) supporting one end of the shaft (24; 26), an axially elongated worm gear (34) extending in a direction generally orthogonal to the shaft (24; 26) and threadably engaging the bracket (32), and drive means (40) for rotatably driving the worm gear (34).

3. Processing apparatus according to claim 1 or 2, wherein the operating means further includes rack and pinion means (44, 46, 48, 50, 52, 54) arranged at opposite ends of the shaft (24; 26) for ensuring that the shaft (24; 26) advances across the support surface (16) while maintaining a sub-

stantially constant orientation with the support surface (16) throughout the range of movement of each pressure applicator (20, 21).

4. Processing apparatus according to claim 3, wherein the rack and pinion means comprises a gear rack (48, 52) extending generally orthogonal to the shaft (24; 26), and a pinion gear (50, 54) intermeshed with the rack (48, 52) and mounted on the shaft (24; 26).

5. Processing apparatus according to claim 4, wherein the operating means further includes bearing means (56, 58) carried by the shaft (24; 26) for facilitating its movement across the support surface (16).

6. Processing apparatus according to claim 5, wherein the operating means further includes a rail (64, 66) adjacent to the gear rack (48, 52) and fixedly arranged relative to the support surface (16) so that the bearing means (56, 58) carried by the shaft (24; 26) rides on the rail (64, 66).

7. Processing apparatus according to any one of the preceding claims, further including temperature-control elements (70, 72) movable with the pressure applicators (20, 21) and operable upon the reaction pack (12) to change its temperature.

8. Processing apparatus according to claim 7, wherein the support surface (16) is apertured to accommodate temperature-control elements (74) beneath the reaction packs (12).

9. Processing apparatus for transferring a fluid between blister-like chambers in a chemical reaction pack comprising:

a support surface (16) for supporting a plurality of the reaction packs (12) in side-by-side and end-to-end relation relative to each other;

at least one roller (20, 21) supported above the support surface (16) and in alignment with the blister-like configurations on the reaction packs (12); and

drive mechanism means (22, 24, 26, 32, 34, 36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 64, 66) for moving the rollers (20, 21) across the support surface (16) to operate against the reaction packs (12) and facilitate transfer of fluid therethrough;

characterized in that the drive mechanism means (22, 24, 26, 32, 34, 36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 64, 66) comprises at least two spaced apart generally parallel shafts (24, 26) each having a series of the rollers (20, 21) arranged endwise thereon;

and in that the drive mechanism means (22, 24, 26, 32, 34, 36, 38, 40, 44, 46, 48, 50, 52, 54, 56, 58, 64, 66) advances the shafts (24, 26) with the rollers (20, 21) arranged thereon across the support surface (16) while maintaining a substantially constant orientation relative to the support surface (16).

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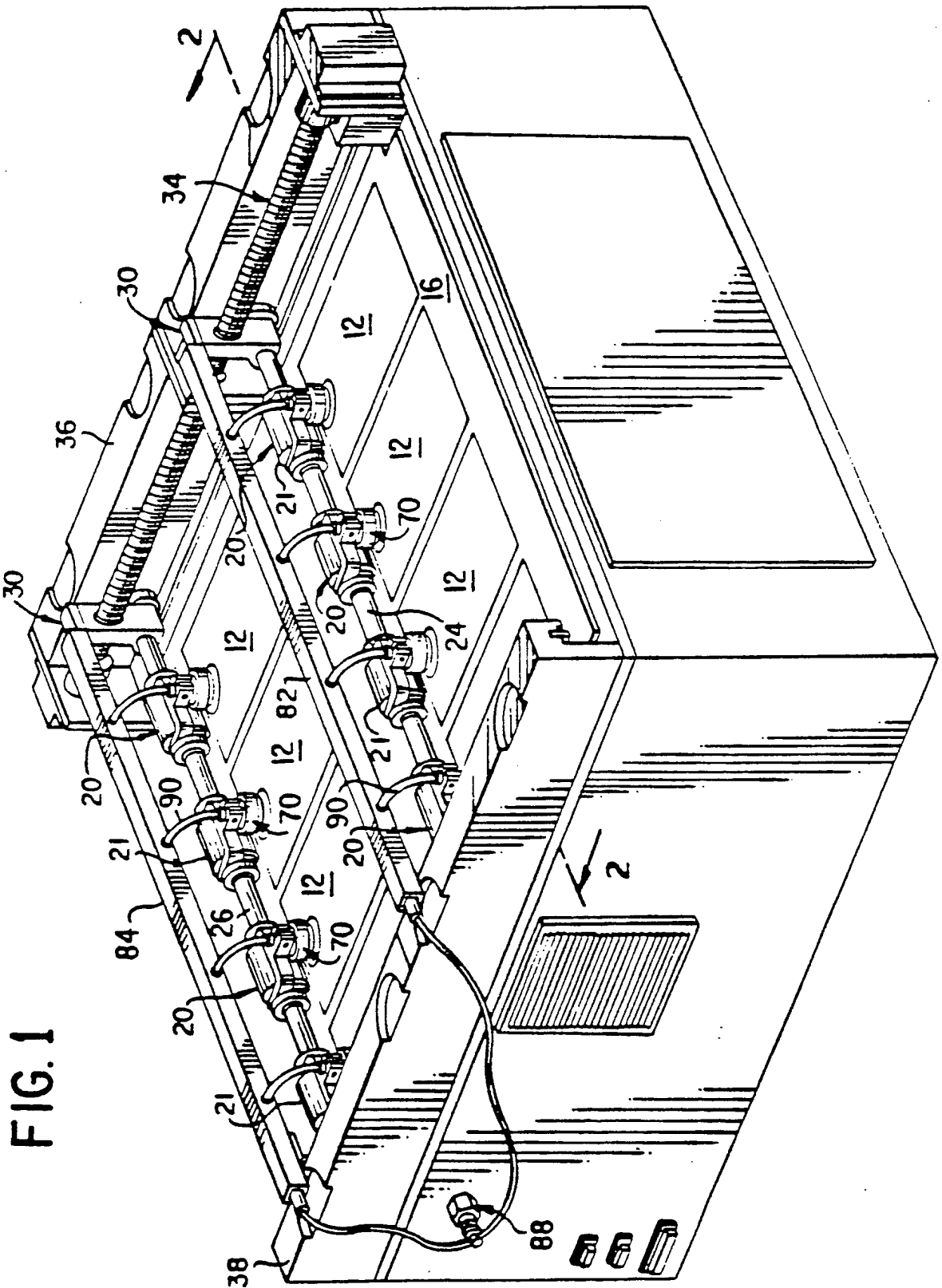
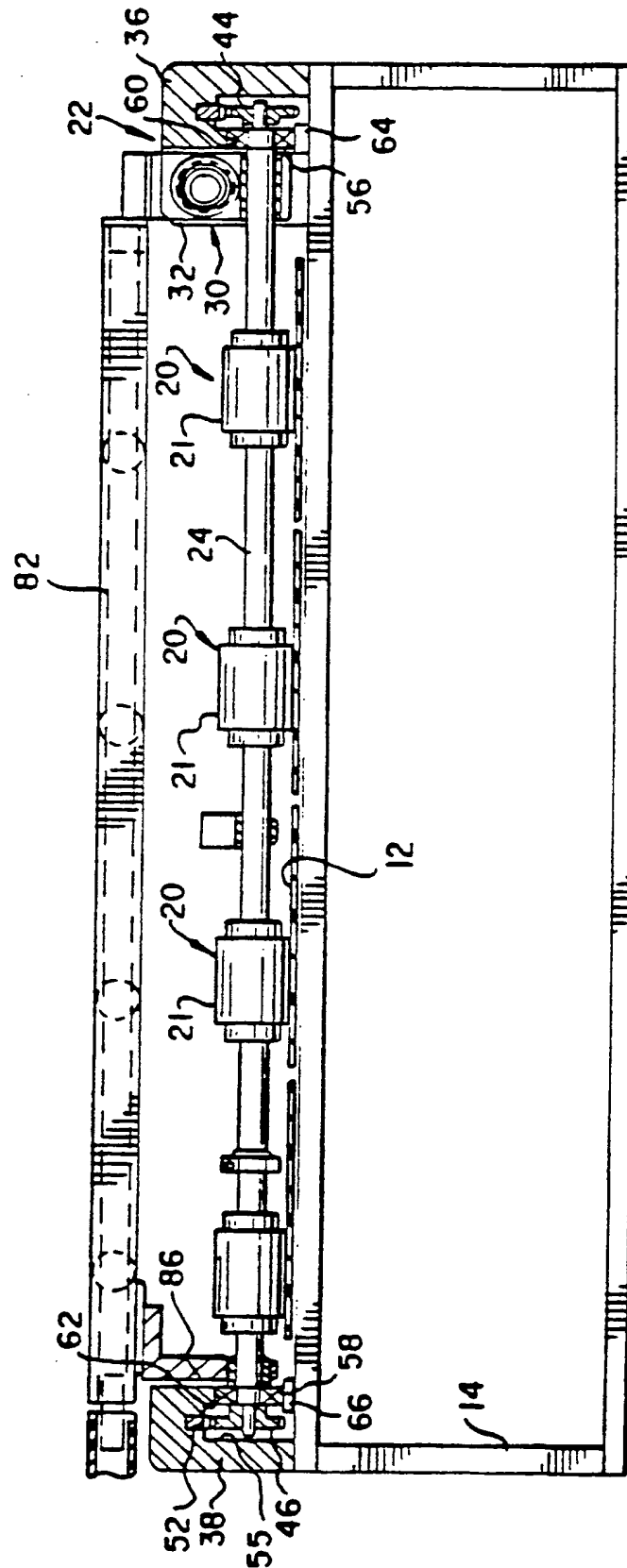


FIG. 1

FIG. 2





**FIG. 3**

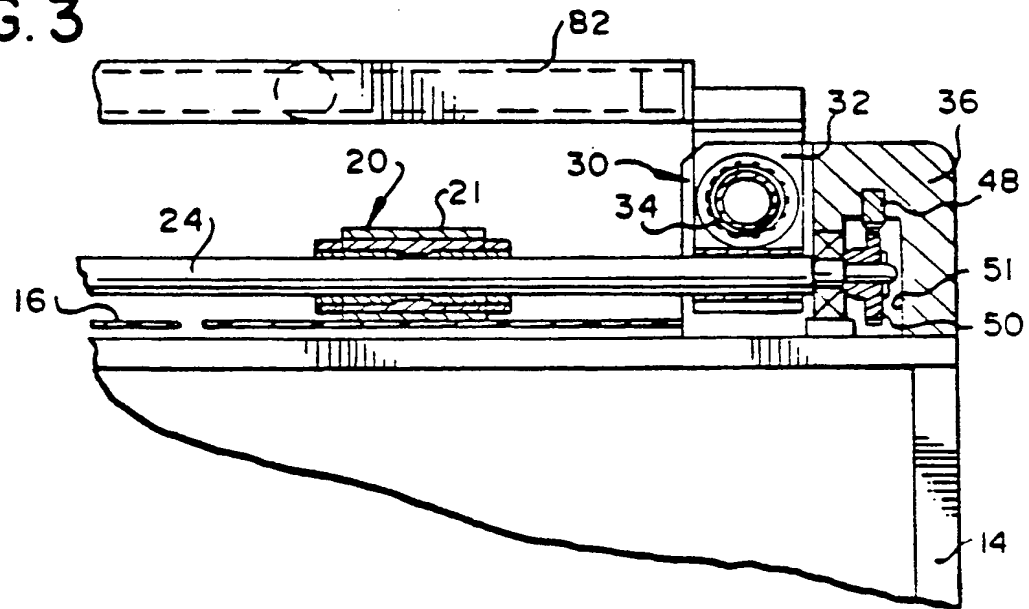


FIG. 5

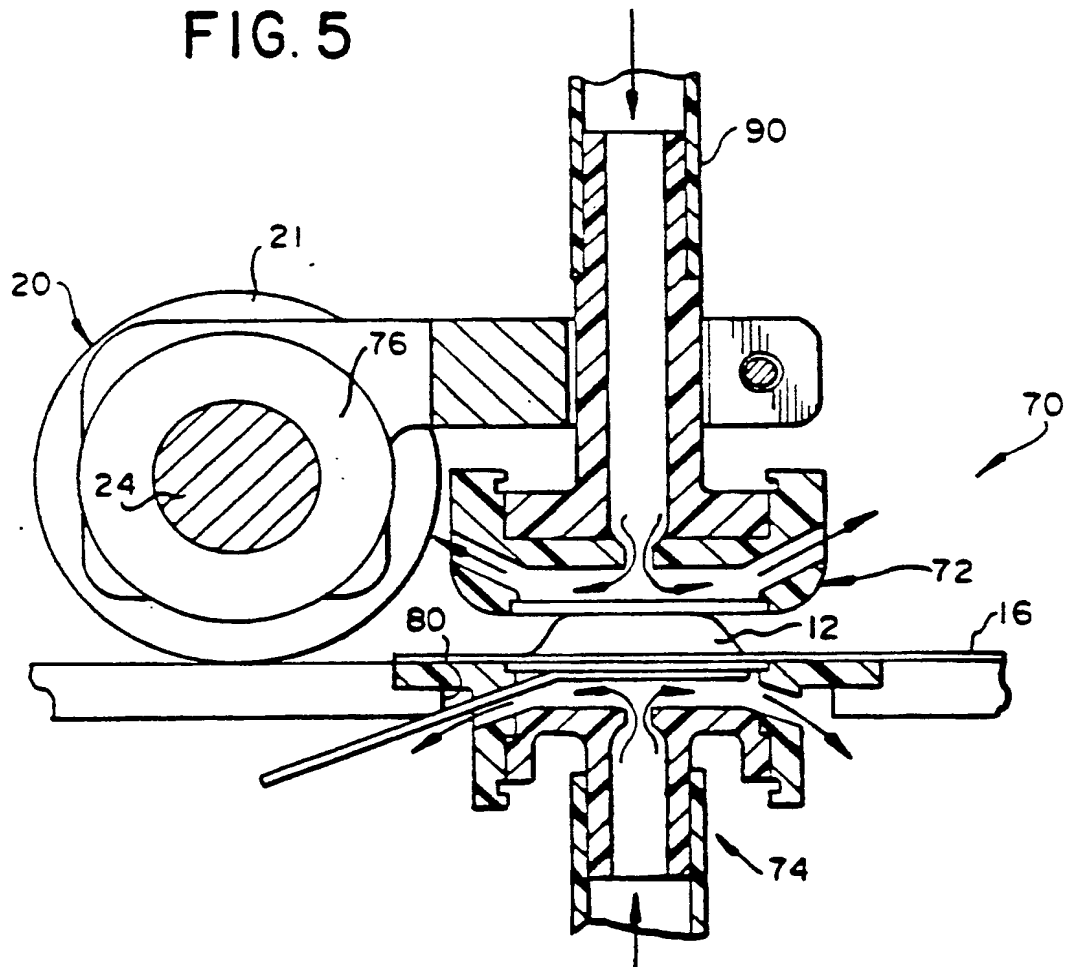
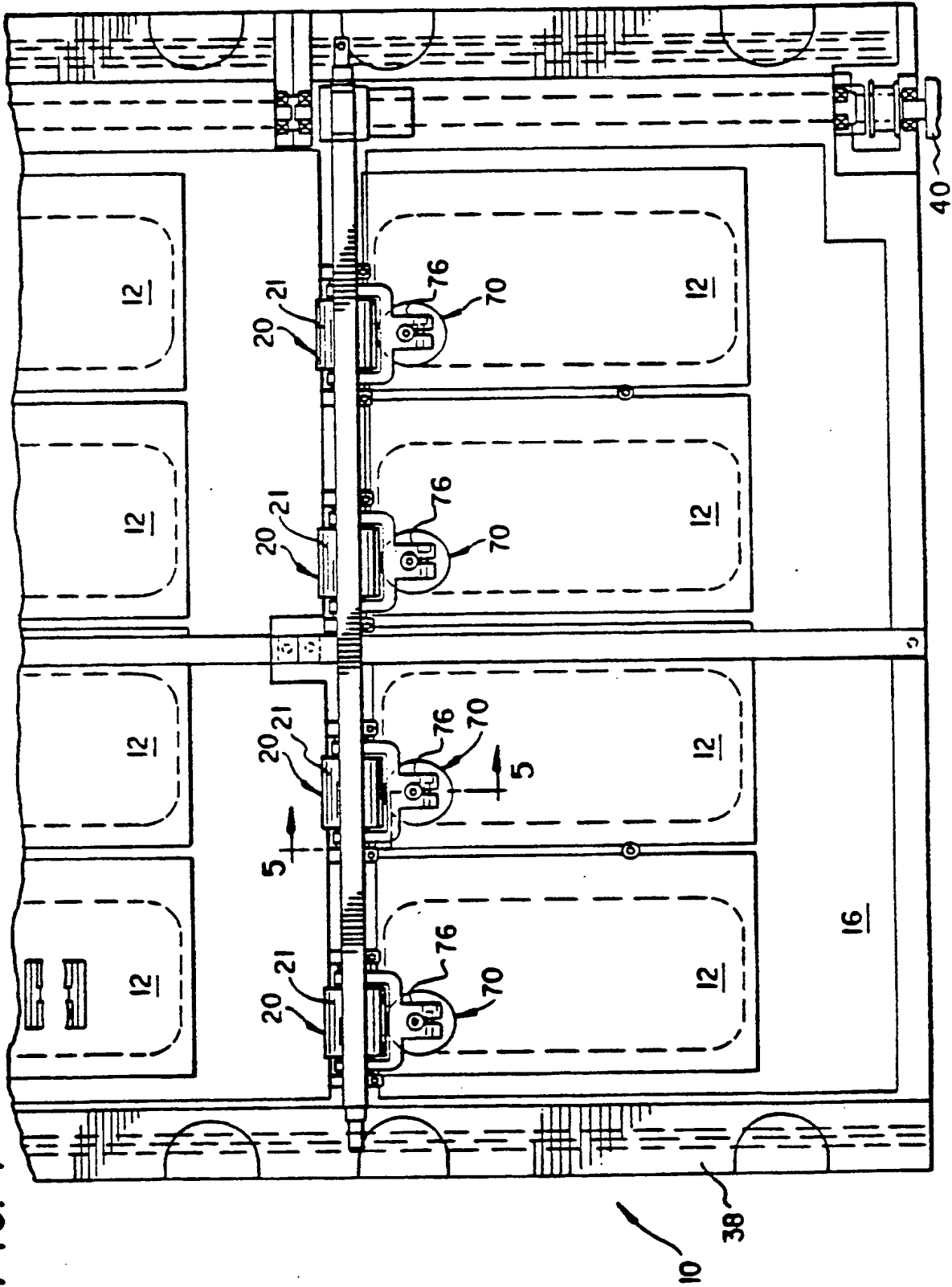


FIG. 4





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(54) **Processing apparatus for a chemical reaction pack.**

(57) Disposable reaction packs for use in automatic analysis equipment are known. These packs comprise a body which is divided into successive chambers, each of which contains a fluid. Mixing of the fluids in such packs is achieved by manually applying pressure to each chamber of the reaction pack to open the seal between each chamber. This process is time-consuming, inaccurate and could result in damage to the reaction pack. Described herein is

apparatus (10) for transferring liquid from one chamber to another in successive chambers of a chemical reaction pack (12). The apparatus (10) comprises a support surface (16) on which the packs (12) are placed, and a plurality of pressure applicators (20) which are driven to apply pressure to the packs (12). Temperature control elements (70) are also provided to heat and cool the liquid contained within each chemical reaction pack (12).

EP 0 402 994 A3



European  
Patent Office

## EUROPEAN SEARCH REPORT

Application Number

EP 90 20 1463

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	US-A-4 236 806 (HOADLEY) * Figures 1-8; column 1, line 41 - column 2, line 7; column 4, lines 29-68 *	1,3	G 01 N 35/06 B 01 L 7/00 C 12 Q 1/68
Y		9	
A		7,8	
Y	FR-A-2 612 295 (G. KARCHER et al.) * Page 2, lines 3-26; page 3, lines 19-34; figures 1,2 *	1,3-6,9	
Y	US-A-2 455 125 (E.H. LAND) * Figures 1,2; column 1, lines 12-32; column 3, line 11 - column 4, line 8; column 4, lines 54-64; column 8, lines 29-37; column 9, lines 9-18 *	1,3-6	
A,D	US-A-3 036 894 (J.A. FORESTIERE)		
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			B 01 L G 01 N G 03 B G 03 D
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		21 May 91	HODSON C.M.T.
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

## Thermopneumatically actuated microvalves and integrated electro-fluidic circuits

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### Abstract

An electro-fluidic multi-chip module (E/F MCM) technology incorporating microfabricated sensors and actuators has been developed for diverse research and commercial applications. The first E/F MCM incorporates a thermopneumatically actuated valve, a piezoresistive pressure sensor, and feedback electronics to create a complete electronically programmable pressure regulator. Both the operation of the valve and the pressure regulator are described. By routing chemicals between various reaction vessels and detectors, future E/F MCM's may be used to process chemical information in a manner analogous to the way computers manipulate electronic information.

### Introduction

The idea of using silicon as an electro-fluidic substrate for chemical analysis evolved in the laboratory of Professor James B. Angell at Stanford University in the late 1970's and early 1980's<sup>1</sup>. In that same laboratory, other microfluidic devices were built<sup>2,3,4</sup> and the first complete integrated microvalve was produced<sup>5,6</sup>. Later, the concept of using silicon as a substrate for multiple valves, electronics, and detectors to perform chemical operations as complex as DNA analysis evolved<sup>7,8,9</sup>. Others have built micropumps<sup>10,11,12</sup>, microvalves with a variety of actuation mechanisms<sup>13,14,15</sup>, flow sensors<sup>16</sup>, and electrophoresis separators<sup>17</sup>. A nice review paper documenting the surge of interest in microfluidics has recently been published<sup>18</sup>. This paper focusses on thermopneumatically-actuated microvalves and their role in integrated circuits that have fluidic as well as electronic elements, enabling chemical instrumentation of increasing complexity but decreasing size.

### Principles of Operation

An Electro-Fluidic Multi-Chip Module (E/F MCM) may be comprised of five functional groups: 1) a fluid management system including flow channels, valves, mixers, and reaction chambers; 2) one or more sensors for measuring any of a number of parameters, including pressure, flow, pH, optical properties, and electrical properties; 3) separation systems for refining chemical products from reagents; 4) interface and control electronics; and 5) a multi-chip module manufacturing technique that integrates these electronic and fluidic functions. In the example of an E/F MCM described herein, the fluid management system is comprised of a thermopneumatic-microfabricated valve, an orifice, input and output ports, and a flow channel connecting them; the sensor is a pressure sensor; the separation system is omitted; the electronics are an analog feedback control circuit that provide pressure regulation; the MCM manufacturing technique is a direct extension of standard PC board manufacturing methods used in the IC industry.

### Operation of the microfabricated valves

The key component of a microfabricated thermopneumatically actuated valve is a silicon diaphragm made by etching a precisely controlled recess in a silicon substrate. A Pyrex wafer with a resistor fabricated onto its surface covers the liquid-filled recess, creating a hermetically sealed control cavity. Dissipating energy in the resistor heats the liquid, increasing the pressure inside of the sealed vessel, increasing the volume of the vessel. This pressure is used to modulate the flow of either gas or liquid in the adjacent channel. In a Normally Open (NO) configuration (Figure 1), the edges of the diaphragm are fixed in space. Increasing control cavity volume pushes the silicon membrane towards the valve seat. In a Normally Closed (NC) configuration (Figure 2), a point on the diaphragm is fixed in space. Increasing control cavity volume causes the edges of the diaphragm to move away from the valve seat. The devices shown in Figures 1 & 2 are both designed for gaseous operation. Similar devices optimized for liquid operation prevent the liquid from contacting the electrical connections.

Since the NO and NC valves are made using a similar process, only the Normally Closed process is illustrated in Figure 3.

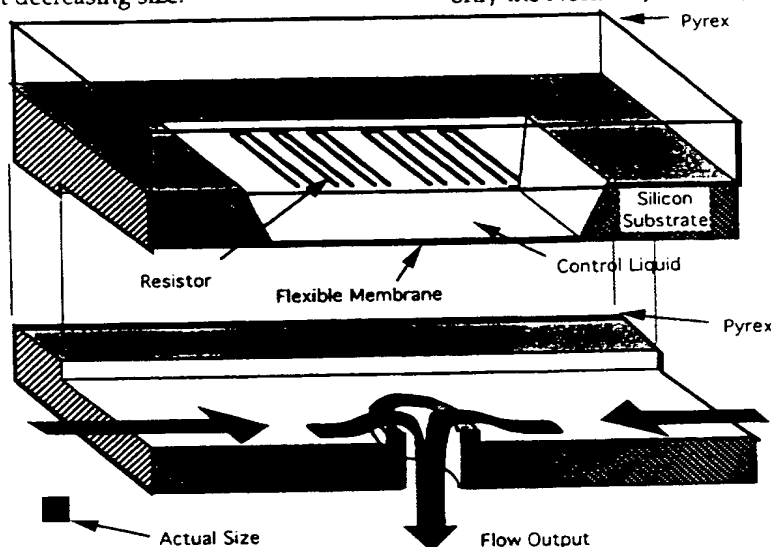


Figure 1: The Normally Open thermopneumatically actuated Fluistor™ Microvalve features a liquid filled cavity which when heated, forces a silicon diaphragm outward over valve seat.

These drawings represent an illustrative cross-section through a single device; typically nearly one hundred devices are made simultaneously on a single wafer. Figure 3a) shows the top pyrex wafer after one side has been coated with 1000Å Pt and patterned into a resistor. It also shows an electric / fluidic via through the pyrex. Figure 3b) shows the first step in the processing of the silicon wafer. Here a thick (1.2 µm) layer of SiO<sub>2</sub> is grown in a humid environment at 1100°C and photolithographically patterned as shown. Figure 3c) shows the silicon wafer after it has been etched in a hot (80°C) solution (33% w/w) of KOH, defining the control cavity and membrane. A second lithography and etch is performed, leaving a 40 µm thick silicon membrane and sealing ring, as shown in Figure 3d). The bottom wafer, shown in Figure 3e) is made of pyrex, which is ultrasonically drilled and polished. Figure 3e) shows the three wafers after they are bonded together. After being filled with the control liquid, Figure 2) illustrates how the cap seals the liquid in the control cavity. The complete valve, pictured in Figure 4, measures 6.3 x 6.6 x 2.0 mm.

One of the advantages of thermopneumatic actuation over other micro-actuated mechanisms — such as thermal bimorph, electrostatic or piezoelectric — is the independence of the actuation and the object being actuated. In this case, the object being moved is a diaphragm, whose flexibility, chemical inertness, and other properties can be completely optimized independently of the actuator. The actuator, i.e., the cavity full of gas and or liquid, can also be optimized independently from the membrane for a particular application by adjusting cavity shape, boiling point of the control liquid, quantity of both gas and liquid molecules, and other properties. The dynamic range of the device can thus be quite broad: vacuum pressures to hundreds of atmospheres; gas, liquid, and corrosive fluids; ultra-low (µl/min of gas) to industrial (10's slpm) flow rates. Valves of this type have been built that control liquids at 204 bar (3000 psig). Flow rates of similar valves have been built that exceed 15 slpm of N<sub>2</sub> at 7 bar (103 psid).

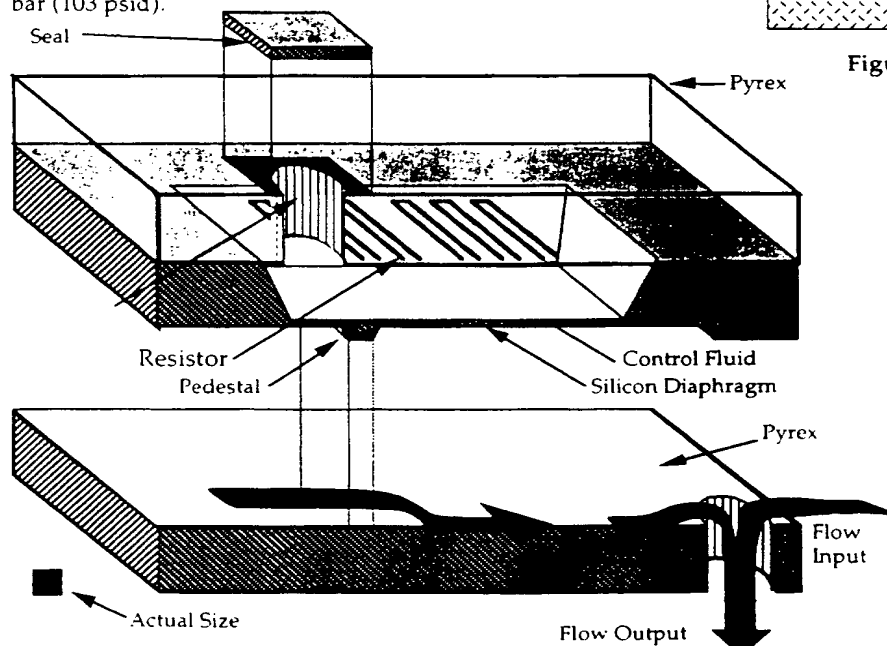


Figure 2: Normally Closed thermopneumatically actuated Fluistor™ Microvalve features a liquid filled cavity which when heated, actuates a silicon diaphragm forcing the valve cover to lift off the valve seat.

Compared to other microvalve technologies, thermopneumatic actuation exerts tremendous force through a long stroke. The maximum displacement of a NO valve is about 50 µm. Since the upper portion of a NC valve rotates, its maximum vertical displacement is about 150 µm. A thermopneumatic actuator is capable of providing significant force throughout this displacement — over 20 N (200 bar x 6900 N/m<sup>2</sup>/bar x 1.6 x 10<sup>-5</sup> m<sup>2</sup>) through a 50 µm stroke has been demonstrated in the case of the NO valve.

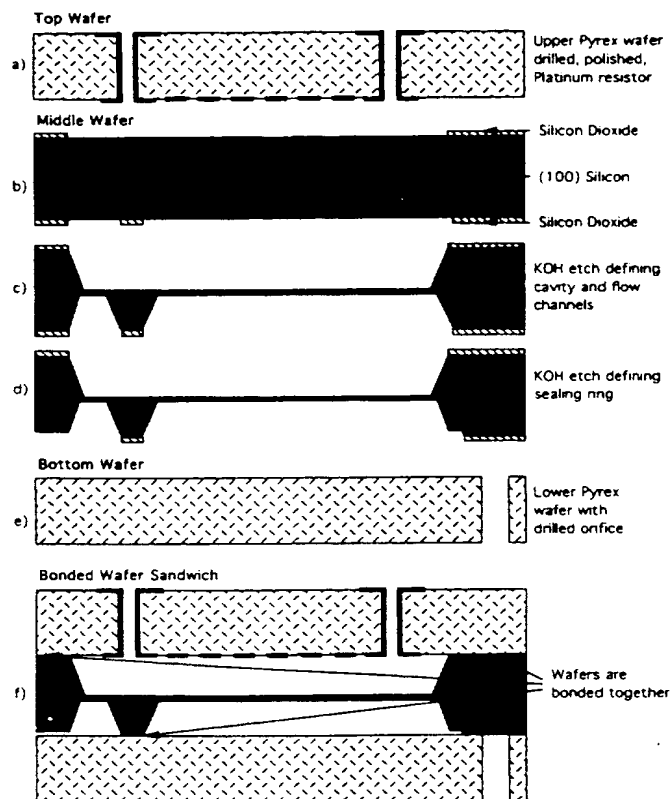


Figure 3: Process flow of a normally closed valve



Figure 4: Photograph of a Fluistor™ microvalve.

This long stroke allows a thermopneumatically-actuated microvalve to control high flow rates and pressures relative to other microvalve technologies. The high operating pressure capability not only extends its application to high pressure applications, it allows the use of significant force for preventing leaks across the valve seat. Long stroke and smooth actuation allows stable control of flow rate over a wide dynamic range: single valves are capable of controlling stable flow rates continuously from 0.001 sccm to 10 slpm. Flow v. pressure at a variety of ambient temperatures and flow v. applied power at a variety of supply pressures are shown in Figure 5a & 5b (Normally Open) and Figure 6a & 6b (Normally Closed).

#### Pressure Sensor

The sensor used in this E/F MCM is a piezoresistive, silicon membrane device (SenSym, Milpitas CA). The silicon sensor is encapsulated in a plastic "button" package that facilitates PC-board mounting. The sensor has a linearity and accuracy of about 1%. Linearity and precision may be extended to about 0.1% through the use of well-established digital compensation techniques that correct for nonlinearities in the sensor caused by temperature and pressure variations.

#### Feedback electronics

The analog control circuit provides the necessary power signal for the pressure sensor and conditions the sensor output appropriately for the implementation of the pressure control system. The analog pressure control system calculates a valve control signal as the sum of three terms; one term proportional to the pressure error, one term proportional to the integral of the pressure error, and one term proportional to the filtered derivative of the pressure error. Therefore, the controller is a classical PID (proportional-integral-derivative) controller. Since the system is non-linear, however, the gains have been set to maximize the time of response without unduly sacrificing transient overshoot or steady-state performance. The selected gains result in a controller which closely resembles a modified-bang-bang controller: bang-bang control until the operating point is within range of linear behavior at which point the PID control behavior dominates. The output of the control electronics is a 0 - 15 Volt signal which serves as the command to the valve chip drive electronics. As the valve membrane deflects due to changes in the voltage applied to the heater, the flow through the valve will vary, changing the sensed pressure, which is used by the controller to continually adjust the valve heater voltage until the sensed pressure matches the desired pressure.

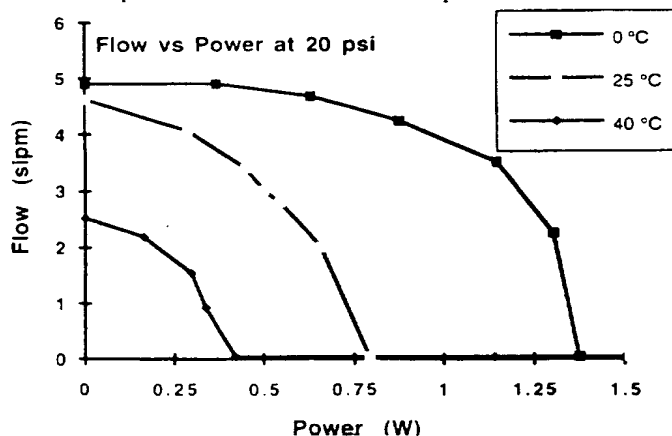


Figure 5a: Flow vs. Power for a Normally Open valve at 20 psig supply pressure and a variety of ambient temperatures.

#### E/F MCM Manufacturing technique

Multi-Chip Module is a widely known manufacturing technique in the electronics industry for integrating electronic chips together at the die-level to reduce the volume of the package and improve the performance of the system. Non-packaged silicon die are bonded to a substrate, which provides electrical connections. In this way, a system designer has the flexibility of purchasing functionally specialized components or systems from different vendors and integrating them in an optimal way. Lower system cost, smaller size, greater reliability, higher frequency response, and electro-optic transduction are all attributes that make MCMs higher value-added products.

Here, we demonstrate an MCM for electro-fluidic circuits, i.e., consists of fluidic and electronic elements. The construction and choice of materials of the fluidic element has an influence on the performance of the valve in the module. Since currently available micromachined valves are thermal devices, the thermal mass and heat conduction of the manifold materials plays an important role in the response time and stability of the system. Ideally, the package should be a perfect heat sink, i.e. the chip interface stays near ambient temperature while dissipating heat from the chip.

Since fluidic interfaces (input/output ports) for E/F MCMs must conform to industry standard fluidic fittings and tubing, these interfaces prevent shrinking the modules even further. For high density packaging requirements, a manifold with flange seal type O-rings is often the best choice. This format can eliminate fittings and tubing since the module can be pressed directly against larger manifolds or instrument bulkheads. In some applications, in-line connections with tube fittings are required — a solution that allows easy interface but sacrifices size efficiency.

The MCM substrate contains milli-flow channels that are used to interconnect various fluidic and electro-fluidic components. This E/F MCM uses three such components: the microvalve, the pressure sensor, and an optional microfabricated capillary or orifice for relieving pressure applications. These three devices are interconnected with a channel buried in the substrate. Electronic traces are patterned on the surface of the substrate using standard PC-Board manufacturing techniques. The first example of an E/F MCM to use both microactuators and microsensors is shown in Figure 7 — the Mini Pressure Regulator.

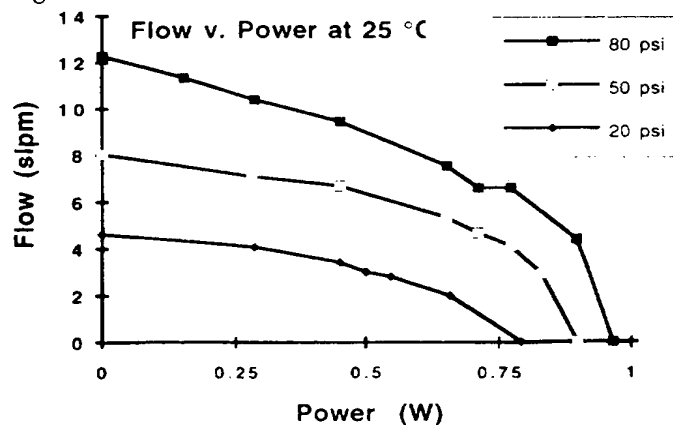


Figure 5b: Flow vs. Power for a Normally Open valve at a variety of supply pressures and 20 °C ambient temperature.

Pressure regulators built using E/F MCM technology have excellent performance characteristics including electronic pressure programmability, broad dynamic range of flow (100,000:1), excellent supply pressure rejection (>100dB) and flow rate rejection (<0.1% full scale pressure/full scale flow), temperature insensitivity (<0.1% full scale pressure/°C), minimal ripple (0.01% full scale pressure), and long term stability (variations <0.1% full scale pressure/year). When compared with manual mechanical regulators, these performance characteristics are striking. Mechanical devices typically have small dynamic range (100:1), less supply pressure rejection (<40 dB), lower flow rate rejection (0.5% full scale pressure/full scale flow rate), reduced temperature insensitivity (1.0% full scale pressure/°C), and poor long term stability (variations greater than 1.0% full scale pressure/year). Although small in size — measuring 6.3 x 8.6 x 1.2 cm — it is capable of regulating continuously over a range of 0 to 100 psig with 0.5% accuracy. The full performance specifications are summarized in Table 1.

Virtually all of the closed-loop performance limitations of the E/F MCM pressure regulator arise due to the sensor calibration limitations. Higher performance E/F MCM pressure regulators will be based on digital control electronics and will incorporate accurately calibrated pressure sensors. Off-the-shelf pressure sensors with minimal compensation yield pressure signals which are accurate to no better than 1.0% full scale pressure. Advanced digital calibration techniques will result in a pressure sensor with accuracies of at least  $\pm 0.1\%$  full scale pressure over a wide range of temperatures. Incorporation of a microprocessor for control opens up the possibilities of using intelligent control algorithms which can more easily implement nonlinear functions making fuzzy logic and neural network-based controllers attractive. Further, the use of high accuracy pressure sensors combined with a microprocessor makes the development of a flow regulator a simple extension of the pressure regulator.

One of the key advantages of the E/F MCM technology is the efficient use of space. Since these modules can be made compactly, individual units can be combined to form more complex systems without sacrificing cost or size over specifically dedicated designs. For example, a manifold of pressure regulators can be efficiently constructed by mounting numerous E/F MCM pressure regulators to a common input gas line. This type of configuration is required by numerous industries including analytical instrumentation,

gas chromatography, and other mixing applications. More complex modules for regulating pressure and flow of gases and liquids will be developed offering greatly reduced size, lower cost and higher performance than systems utilizing mechanical valves.

#### Application of E/F MCM's in Medical Instrumentation

The trend in the Medical marketplace is constantly towards reducing size and costs. Medical applications more commonly involve carrying liquids not gases and require on-off switching rather than proportional control. For these applications, E/F MCM materials require a higher degree of corrosion resistance and the feedback circuitry must close the control loop very quickly.

There are numerous medical applications where current E/F MCMs can perform proportional control of gas pressure. Anesthesia machines can be upgraded to perform automatic gas mixing and flow control — functions which today are still performed with manual valves and regulators. Insufflators, instruments which inflate the abdominal cavity during surgery, can be equipped with Micro Pressure Regulators to provide dynamic closed-loop control of pressure during surgery, replacing larger, more expensive and lower performance electro-mechanical valve-based systems. For Non-Invasive Blood Pressure monitoring (NIBP), E/F MCMs can be used to rapidly inflate and deflate pressure cuffs in a stable and predictable manner to facilitate rapid and accurate blood pressure measurement — the size and affordability of the E/F MCMs will enable the NIBP industry to utilize closed-loop control of pressure to replace unstable, open-loop solenoid valve systems.

Future E/F MCM's designed for liquid operation could provide a new platform on which a variety of clinical chemistry operations could be performed. A concept sketch of such a "Universal chemical processor" is shown in Figure 8. Such a processor would have a number of different elements: an array of input ports to connect to a supply of reagents and samples; an array of valves to route selected chemicals to and from various nodes; a number of temperature controlled reaction chambers where the chemical reactions would take place; an array of separation devices for separating certain chemicals from each other; an array of detectors that sense the presence of chemical products; an array of output ports for disposing of waste materials as well as any chemicals that have been synthesized. A number of these microfabricated elements have been developed by various researchers around

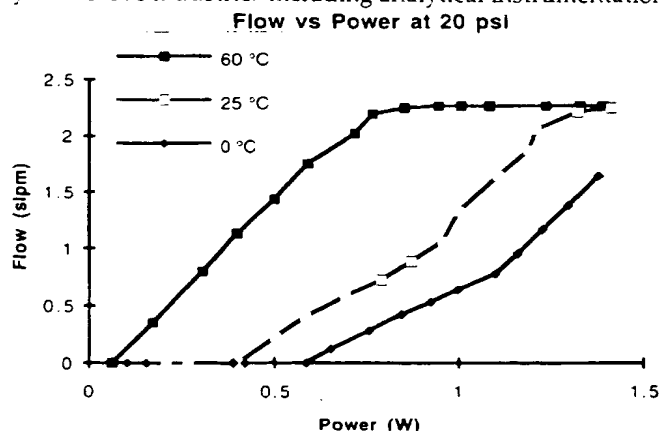


Figure 6a: Flow vs. Power for a Normally Closed valve at 20 psig supply pressure and a variety of ambient temperatures.

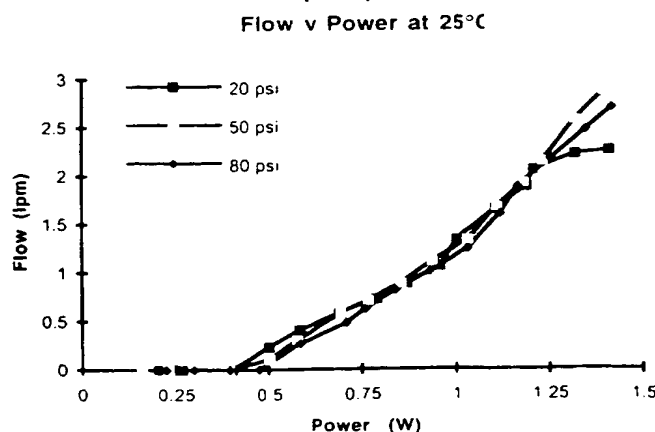


Figure 6b: Flow vs. Power for a Normally Closed valve at a variety of supply pressures and 20 °C ambient temperature.



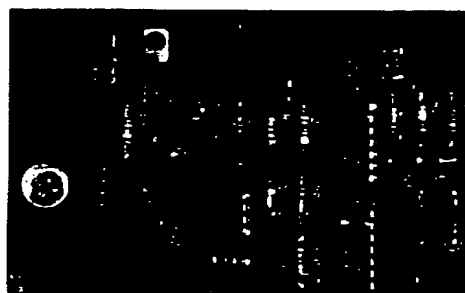
the world, including electrophoresis separation, optical detectors, pH sensors, valves, and temperature controlled reaction chambers. Integrating them together will provide a compact chemical analysis system with tremendous flexibility and sensitivity.

### Conclusions

A microfabricated valve has been developed for operation on both gases and liquids. This device has enabled the development of Electro/Fluidic Multi-Chip-Modules. Already, this level of integration provides an architecture capable of perform complex functions in a small space. The next challenge will be to provide these chips in a monolithic construct, with multiple valves or sensors manufactured simultaneously on a common substrate — the first Electro-Fluidic Integrated Circuits. Mirroring the semiconductor industry, these chips will rapidly increase in capabilities, as their size and costs are reduced. The revolutionary impact on the instrumentation marketplace will be analogous to the impact of semiconductors on the computer marketplace. Today's instruments — the equivalent of yesterday's mainframe computers — will be replaced by the instrumentation equivalents of workstations, lap-tops, and field portable units.

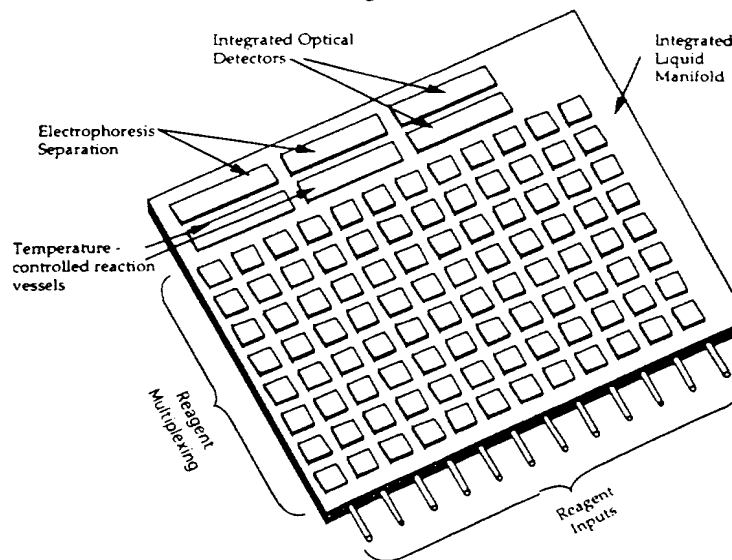
**-TABLE 1-**  
Performance Benefits of E/F MCM Pressure Regulators

Performance Criteria	E/F MCM	Mechanical
Dynamic Range:	100,000:1	100:1
Supply Rejection:	> 100 dB	< 40 dB
Flow Rate Rejection: (percent of full scale pressure)	< 0.1%	0.5%
Temperature Insensitivity: (percent of full scale pressure/°C)	< 0.1%	1.0%
Long Term Stability: (percent pressure variation/year)	< 0.1%	1.0%
Programmability:	yes	no



**Figure 7:** The size and cost benefits of E/F MCM products increase as product complexity increases. Custom manifolds and multiplexors — such as this pressure regulator for a protein synthesizing instrument — will replace larger, more expensive electro-mechanical systems.

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**Figure 8:** Universal chemical analysis manifold

